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(54) Title: NOVEL ORGANIC ANION TRANSPORT PROTEINS

(57) Abstract: The current invention discloses nucleic acid and amino acid sequences for novel organic anion transfer proteins ("OATPs"). The invention encompasses the OATPs described herein, together with vectors containing the cDNA sequences, host cells containing the vectors and polypeptides having all or part of an OATP. Also encompasses are uses for OATPs for targeting drugs to specific organs and for modulating the concentration of endogenous substrates.

NOVEL ORGANIC ANION TRANSPORT PROTEINS

This application claims priority from provisional U.S. Application Serial No. 60/135,081, filed May 20, 1999, which is incorporated herein by reference in its entirety.

Field of the Invention

The invention claims isolated nucleic acid encoding all or a portion of novel members of the organic anion transport protein ("OATP") designated OATP2, OATP-RP1, OATP-RP2, OATP-RP3, OATP-RP4 and OATP-RP5. Also claimed are vectors containing the nucleic acid sequences, host cells containing the vectors and polypeptides having all or part of the amino acid sequence of OATP2, OATP-RP1, OATP-RP2, OATP-RP3, OATP-RP4 and OATP-RP5. Tissue expression of the transporter is described as well as some of its substrates. Also claimed are uses for these novel OATPs, including for targeting drugs to specific tissues, for modulating the concentration of endogenous substrates, and for identifying a substrate capable of being transported by a novel OATP of the invention.

Background of the Invention

The liver functions in the clearance of a large variety of metabolic products, drugs and other xenobiotics by transporting them across the sinusoidal membrane into the hepatocyte. Several classes of transport systems have been described that mediate these processes including the Na+/taurocholate cotransporter polypeptide, NTCP, in rat and human liver (Hagenbuch, B., et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:10629-33; Hagenbuch, B. et al., (1994) *J. Clin. Invest.* 93:1326-31) and a family of organic anion transporting polypeptides (OATPs) that are principally expressed in liver, kidney and brain, and transport a broad spectrum of substrates in a sodium-independent manner (Meier, P.J., et al., (1997) *Hepatology* 26:1667-77; Wolkoff, A.W., (1996) *Semin. Liver Dis.* 16:121-127). The distribution of this latter family of

transporters in liver, kidney and choroid plexus in the brain is thought to reflect common physiological requirements of these organs for the clearance of a multitide of organic anions. There are three OATP isoforms in the rat: roatp1 (Jacquemin, E., et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:133-37); roatp2 (Noe, B.A., et al., (1997) *Proc. Natl. Acad. Sci. USA* 94:10346-50; and roatp3 (Abe, T., et al., (1998) *J. Biol. Chem.* 273:11395-401). In addition to bile acids, OATPs are known to transport a variety of other compounds. These include, depending on the transporter, unconjugated and conjugated steroids such as estrone sulfate, estradiol-17B-glucuronide, aldosterone, and cardiac glycosides (Boussuyt, X., et al., (1996) *J. Pharmacol. Exp. Ther.* 276:891-6; Boussuyt, X. (1996) *J. Hepatol.* 25:733-8; Kanai, N., et al., (1996) *Am. J. Physiol.* 270:F319-F325; Kanai, N., et al., (1996) *Am. J. Physiol.* 270:F326-F331; Noe, B.A., et al., (1997) *Proc. Natl. Acad. Sci. USA* 94:10346-50). Bromosulfophthalien (Jacquemin, E., et al., (1994) *Proc. Natl. Acad. Sci. USA* 91:133-7); mycotoxin (Kontaxi, M., et al., (1996) *J. Pharmacol. Exp. Ther.* 279:1507-13); leukotriene C_4 (Li, L., et al., (1998) *J. Biol. Chem.* 273:16184-91); and thyroid hormone (Abe, T., et al., (1998) *J. Biol. Chem.* 273:11395) are additional substrates.

Several proteins have been identified. Jacquemin,E., et al., (1994) *Proc. Natl. Acad. Sci. U.S.A.*, 91:133-137 reported the first cloning and identification of a member of the OATP transporter family, namely the rat oatp1. The first cloning and identification of a human OATP was reported in Kullak-Ublick, G.A., et al., (1995) *Gastroenterology*, 109:1274-1282. Its expression was found in liver, kidney brain and other organs. The authors concluded, based on substrate specificities, that it was not the human orthologue of rat oatp1.

Substrate specificities of rat oatp1 are discussed in Kullak-Ublick, G.A. et al., (1994) *Hepatology*, 20:411-416, while substrate specificities of human OATP are discussed in Bossuyt, X., et al., (1996) *J. Hepatol.*, 25:733-738.

Data was later discovered showing that rat oatp1 is involved in the transport of steroids (Bossuyt, X., et al., (1996) *J. Pharmacol. Exp. Ther.*, 276:891-896), and that human OATP acts as a transporter for the psychoactive hormone DHEAS (Kullak-Ublick, G.A., et al., (1998) *FEBS Lett.*, 424:173-176). For a review of the OATP

family and organic anoin transport in the liver, see Wolkoff, A.W., (1996) *Semin. Liver Dis.*, 16:121-127.

A third rat OATP isoform that was shown to transport thyroid hormones T3 and T4 was cloned and reported in Abe,T., et al., (1998) *J. Biol. Chem.*, 273:22395-22401.

All references cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

Summary of the Invention

The present invention encompasses novel organic anion transport proteins ("OATP") and polynucleotides encoding said OATPs. The OATPs disclosed herein are designated OATP2, OATP-RP2, OATP-RP3, OATP-RP4, OATP-RP5 and OATP-RP1. A polynucleotide sequence of each OATP is disclosed herein, along with the deduced amino acid sequence. The cDNAs encoding the OATPs of the present invention have been deposited with the American Type Culture Collection and given Accession Numbers ATCC 207213 (OATP2), ATCC 207212 (OATP-RP2), ATCC 207209 (OATP-RP3), ATCC 207210 (OATP-RP4), ATCC 207211 (OATP-RP5), and ATCC 207214 (OATP-RP1).

The present inventors sequenced the cDNAs encoding the novel OATPs and determined the primary sequence of the deduced proteins. Disclosed herein are the nucleic acid sequence (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of OATP2; the nucleic acid sequence (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of OATP-RP2; the nucleic acid sequence (SEQ ID NO:5) and amino acid sequence (SEQ ID NO:6) of OATP-RP3; the nucleic acid sequence (SEQ ID NO:7) and amino acid sequence (SEQ ID NO:8) of OATP-RP4; the nucleic acid sequence (SEQ ID NO:9) and amino acid sequence (SEQ ID NO:10) of OATP-RP5; and the nucleic acid sequence (SEQ ID NO:11) and amino acid sequence (SEQ ID NO:12) of OATP-RP1.

The OATPs of the present invention can be produced by: (1) inserting the cDNA of a disclosed OATP into an appropriate expression vector; (2) transfecting the expression vector into an appropriate transfection host(s); (3) growing the transfected

host(s) in appropriate culture media; and (4) assaying the transport activity in the transfected cells.

The present invention therefore provides a purified and isolated nucleic acid molecule, preferably a DNA molecule, having a sequence which codes for an OATP, or an oligonucleotide fragment of the nucleic acid molecule which is unique to an OATP of the invention. In a preferred embodiment of the invention, the purified and isolated nucleic acid molecule has the sequence as shown in SEQ ID NO:1 (OATP2). In another preferred embodiment, the purified and isolated nucleic acid molecule has the sequence as shown in SEQ ID NO:3 (OATP-RP2). In still another preferred embodiment the purified and isolated nucleic acid molecule has the sequence as shown in SEQ ID NO:5 (OATP-RP3). In still another preferred embodiment of the present invention the purified and isolated nucleic acid molecule has the nucleotide sequence as shown in SEQ ID NO:7 (OATP-RP4). In still another preferred embodiment the purified and isolated nucleic acid molecule has the sequence as shown in SEQ ID NO:9 (OATP-RP5). In still another preferred embodiment of the present invention the purified and isolated nucleic acid molecule has the nucleotide sequence as shown in SEQ ID NO:11 (OATP-RP1).

The invention also contemplates a double stranded nucleic acid molecule comprising a nucleic acid molecule of the invention or an oligonucleotide fragment thereof hydrogen bonded to a complementary nucleotide base sequence.

The terms "isolated and purified nucleic acid", "isolated and purified polynucleotide", "substantially pure nucleic acid", and "substantially pure polynucleotide", e.g., substantially pure DNA, refer to a nucleic acid molecule which is one or both of the following: (1) not immediately contiguous with either one or both of the sequences, e.g., coding sequences, with which it is immediately contiguous (i.e., one at the 5' end and one at the 3'end) in the naturally occurring genome of the organism from which the nucleic acid is derived; or (2) which is substantially free of a nucleic acid sequence with which it occurs in the organism from which the nucleic acid is derived. The term includes, for example, a recombinant DNA which is incorporated into a vector, e.g., into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment

produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. Substantially pure or isolated and purified DNA also includes a recombinant DNA which is part of a hybrid gene encoding additional OATP sequence.

The present invention provides in one embodiment: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding all or a portion of a protein having the amino acid sequence as shown in SEQ ID NO:2 (OATP2); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which exhibit at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The degree of homology (percent sequence identity) between two sequences may be determined, for example, by comparing the two sequences using computer programs commonly employed for this purpose. One suitable program is the GAP computer program described by Devereux et al., (1984) *Nucl. Acids Res.* 12:387. The GAP program utilizes the alignment method of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:433, as revised by Smith and Waterman (1981) *Adv. Appl. Math.* 2:482. Briefly, the GAP program defines percent identity as the number of aligned symbols (i.e., nucleotides or amino acids) which are identical, divided by the total number of symbols in the shorter of the two sequences.

As used herein the term "stringent conditions" encompasses conditions known in the art under which a nucleotide sequence will hybridize to: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding a protein having the amino acid sequence as shown herein, or to (b) a nucleic acid sequence complementary to (a). Screening polynucleotides under stringent conditions may be carried out according to the method described in Nature, 313:402-404 (1985). Polynucleotide sequences capable of hybridizing under stringent conditions with the polynucleotides of the present invention may be, for example, allelic variants of the disclosed DNA sequences, or may be derived from other sources. General techniques of nucleic acid hybridization are disclosed by Sambrook et al., "Molecular Cloning: A Laboratory Manual", 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor,

New York (1984); and by Haymes et al., "Nucleic Acid Hybridization: A Practical Approach", IRL Press, Washington, D.C. (1985), which references are incorporated herein by reference.

The present invention provides in another embodiment: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding all or a portion of a protein having the amino acid sequence as shown in SEQ ID NO:4 (OATP-RP2); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which are at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention provides in another embodiment: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding all or a portion of a protein having the amino acid sequence as shown in SEQ ID NO:6 (OATP-RP3); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which are at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention provides in another embodiment: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding all or a portion of a protein having the amino acid sequence as shown in SEQ ID NO:8 (OATP-RP4); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which are at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention provides in another embodiment: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding all or a portion of a protein having the amino acid sequence as shown in SEQ ID NO:10 (OATP-RP5); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which are at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention provides in another embodiment: (a) an isolated and purified nucleic acid molecule comprising a sequence encoding all or a portion of a protein having the amino acid sequence as shown in SEQ ID NO:12 (OATP-RP1); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences which are at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention also provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:1 (OATP2); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention further provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:3 (OATP-RP2); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention further provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:5 (OATP-RP3); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention further provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:7 (OATP-RP4); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention further provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:9 (OATP-RP5); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention further provides: (a) a purified and isolated nucleic acid molecule comprising a sequence as shown in SEQ ID NO:11 (OATP-RP1); (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) that is at least 18 bases and which will hybridize to (a) or (b) under stringent conditions.

The present invention additionally covers polynucleotides and amino acid sequences of the present invention having one or more structural mutations including replacement, deletion or insertion mutations. For example, a signal peptide may be deleted, or conservative amino acid substitutions may be made to generate a protein that is still biologically competent or active.

The invention further contemplates a recombinant molecule comprising a nucleic acid molecule of the present invention or an oligonucleotide fragment thereof and an expression control sequence operatively linked to the nucleic acid molecule or oligonucleotide fragment. A transformant host cell including a recombinant molecule of the invention is also provided.

In another aspect, the invention features a cell or purified preparation of cells which include a novel gene encoding an OATP of the present invention, or which otherwise misexpresses a gene encoding an OATP of the present invention. The cell preparation can consist of human or non-human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, non-human primate cells, or pig cells. In preferred embodiments, the cell or cells include an OATP transgene, e.g., a heterologous form of an OATP gene, e.g., a gene derived from humans (in the case of a non-human cell). The OATP transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpresses an endogenous OATP gene, e.g., a gene that expression of which is disrupted, e.g., a

knockout. Such cells can serve as a model for studying disorders which are related to mutated or misexpressed OATP alleles for use in drug screening.

Still further, the invention provides plasmids which comprise the nucleic acid molecules of the invention. Also encompassed within the invention are vectors comprising the nucleic acid sequences disclosed herein, as well as host cells comprising said vectors.

The present invention also includes a novel OATP of the present invention, or an active part thereof. A biologically competent or active form of the protein or part thereof is also referred to herein as an "active OATP or part thereof".

The invention further contemplates antibodies having specificity against an epitope of an OATP of the present invention or part of the protein. These antibodies may be polyclonal or monoclonal. The antibodies may be labeled with a detectable substance and they may be used, for example, to detect a novel OATP of the invention in tissue and cells. Additionally, the antibodies of the present invention, or portions thereof, may be used to make targeted antibodies that destroy OATP expressing cells (e.g., antibody-toxin fusion proteins, or radiolabelled antibodies).

The invention also permits the construction of nucleotide probes which encode part or all of a novel OATP protein of the invention or a part of the protein. Thus, the invention also relates to a probe comprising a nucleotide sequence coding for a protein, which displays the properties of a novel OATP of the invention or a peptide unique to the protein. The probe may be labeled, for example, with a detectable (e.g., radioactive) substance and it may be used to select from a mixture of nucleotide sequences a nucleotide sequence coding for a protein which displays the properties of a novel OATP of the invention.

The present invention also provides a transgenic non-human animal (e.g., a rodent, e.g., a mouse or a rat, a rabbit or a pig) or embryo all of whose germ cells and somatic cells contain a recombinant molecule of the invention, preferably a recombinant molecule comprising a nucleic acid molecule of the present invention encoding an OATP of the invention or part thereof. The recombinant molecule may comprise a nucleic acid sequence encoding an OATP of the present invention with a structural mutation, or may comprise a nucleic acid sequence encoding an OATP of the invention or part thereof and one or more regulatory elements which differ from

the regulatory elements that drive expression of the native protein. In another preferred embodiment, the animal has an OATP gene which is misexpressed or not expressed, e.g., a knockout. Such transgenic animals can serve as a model for studying disorders that are related to mutated or misexpressed OATPs of the present invention.

The invention still further provides a method for identifying a substance which is capable of binding a novel OATP of the invention, comprising reacting a novel OATP of the invention or part of the protein under conditions which permit the formation of a complex between the substance and a novel OATP protein or part of the protein, and assaying for substance-OATP complexes, for free substance, for non-complexed OATP, or for activation of an OATP.

An embodiment of the invention provides a method for identifying substrates which are capable of binding to a novel OATP protein of the invention, isoforms thereof, or part of the protein, said method comprising reacting a novel OATP protein of the invention, isoforms thereof, or part of the protein, with at least one substrate which potentially is capable of binding to the protein, isoform, or part of the protein, under conditions which permit the formation of substrate-transporter protein complexes, and assaying for substrate-transporter protein complexes, for free substrate, for non-complexed OATP protein, or for activation of an OATP. In a preferred embodiment of the method, substrates are identified which are capable of binding to and being transported by a novel OATP protein of the invention, isoforms thereof, or part of the protein.

The invention also provides methods for screening potentially useful pharmacological agonists or antagonists of the OATPs of the present invention. The method comprises testing potential agents by adding the agent to be tested to a cell expressing a novel OATP of the present invention in the presence of a compound known to be transported by an OATP of the invention, and measuring the augmentation or inhibition of transport of the known compound.

An OATP of the present invention is also useful to identify compounds that may be transported into an organ, e.g., the liver. Compounds that are found to be actively transported into the liver are useful as carriers for other therapeutics targeting the liver.

Also included within the scope of the present invention is a composition which includes an OATP of the present invention, a fragment thereof (or a nucleic acid encoding said OATP or fragment thereof) and one or more additional components, e.g., a carrier, diluent or solvent. The additional component can be one that renders the composition useful for in vitro, in vivo, pharmaceutical or veterinary use.

Encompassed within the present invention are agonists and antagonists of an OATP of the present invention. Pharmacological agonists or antagonists are useful to increase or decrease the flow of compounds transported by an OATP of the present invention. Said agonists and/or antagonists of the present invention are preferably administered with an acceptable carrier, diluent or solvent.

In another aspect, the present invention relates to a method of treating a mammal, e.g., a human, at risk for a disorder, e.g., a disorder characterized by aberrant or unwanted level or biological activity of an OATP of the present invention. Additionally, encompassed within the invention is a method of treating a mammal, e.g., a human, at risk for disorders of the liver. Since OATP2 is expressed exclusively in the liver, compounds that are optimized for OATP2 are useful to target hepatic delivery. These compounds in themselves may be useful therapeutics, or may be useful to chaperone other therapeutic compounds to the liver. In addition, blocking OATP2-compound interactions could provide benefit by decreasing its first-pass extraction by the liver and, thus, increasing plasma concentrations and prolonging the systemic half-life of a drug.

Also within the scope of the present invention are fusion proteins comprising all or a portion of an OATP of the present invention.

The primary object of the present invention is the identification of new human OATPs, as identified by the nucleic acid and amino acid sequences disclosed herein. Additional objects of the invention are the methods of using the cDNA, the OATP proteins, monoclonal antibodies specific for the novel OATPs, fusion proteins comprising a portion of the OATP protein of the present invention, and agonists and/or antagonists of the novel OATPs as described above.

Brief Description of the Figures

Figure 1 is a Northern blot showing the mRNA tissue distribution of OATP2, OATP-RP1, OATP-RP2, OATP-RP4, and OATP-RP5. The tissues corresponding to the abbreviations above the lanes are indicated below.

Figure 2 shows that OATP2 transports pravastatin, dehydroepiandosterone sulfate (DHEAS), taurocholate and thyroid hormone (T). Figure 2A shows specific uptake of [^{3}H]-pravastatin and [^{3}H]-DHEAS. Figure 2B shows specific uptake of [^{3}H]-taurocholate. Panel 2C shows specific uptake of [125I]-thyroid hormone (T4). The uptake of radiolabeled substrate for 5 minutes into cells transfected with pCEPOATP-RP1 or empty vector (MOCK) was determined in the absence (solid bars) and presence (open bars) of excess unlabeled substrate.

Figure 3 shows a sequence alignment of OATP family members. The protein sequences of human OATP2, OATP-RP1, OATP-RP2, OATP-RP3, OATP-RP4, and OATP-RP5 are aligned with the other known OATP family members. Also shown is a concensus sequence in bold. A concensus is indicated if at least 6 out of the 12 sequences are identical at a given position. A residue is capitalized if it agrees with the concensus.

Detailed Description of the Invention

The following definitions apply to the terms used throughout this specification, unless otherwise defined in specific instances:

"cloning" - isolation of a particular gene from genetic material, for example a genome, genomic library, or cDNA library into a plasmid or other vector;

"coding region" – the region of a nucleic acid sequence that codes for an active protein;

"OATP" – organic anion transport protein;

"stringent conditions" (as used concerning nucleic acid hybridization)— Southern blotting washed in 0.1 X SSC and 0.1% SDS at a temperature of at least about 65° C. See Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982); one skilled in the relevant art would recognize that less stringent conditions (e.g., 1X or 2X SSC,

0.1%SDS) may be employed in using the novel sequences disclosed herein to identify nucleic acid sequences encoding novel OATPs.

"Northern blotting"—a method of identifying particular RNA fragments by hybridization with a complementary nucleic acid, typically a cDNA or an oligonucleotide;

"open reading frame" or "ORF"—a DNA sequence containing a series of nucleotide triplets coding for amino acids and lacking any termination codes;

"plasmid"—cytoplasmic, autonomously replicating DNA elements found in microorganisms;

"promoter"—a region on DNA at which RNA polymerase binds and initiates transcription; and

"Southern blotting"—a method of identifying particular DNA fragments by hybridization with a complementary nucleic acid, typically a cDNA or an oligonucleotide;

"transport" - the movement of a substance across a biological membrane as determined by measuring the redistribution of such a substance across the membrane upon exposure to a transporter.

For definitions of other terms in this specification, see F. Sherman et al., Laboratory Course Manual for Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1987) and Lewin, B., Genes IV, Oxford University Press, Oxford (1990). For the definitions of abbreviations, see Aldrichimica Acta, Vol. 17, No. 1 (1984).

Use and utility

The amino acid sequences of the novel organic anion transport proteins of the present invention are aligned with known transporters of this family in Figure 3. The degree of sequence homology between the sequences of the present invention and known organic anion transporters indicates that the proteins of the present invention are organic anion transporters.

It is believed by those skilled in the art that OATP proteins may be involved in the transport of compounds into the liver. Persons of ordinary skill in the art can use the OATP proteins of the present invention to assay for agents that may increase or

decrease the rate of transport of compounds into the liver, or for compounds that are transported by the OATPs of the present invention that are useful as carriers for other compounds that are desired to be carried to a specific organ (e.g., the liver).

Therefore, agents that increase or decrease the rate of substrate transport by the OATPs of the present invention, or agents identified as carriers, are useful in the treatment of liver disease.

Because some of the OATPs of the present invention are organ specific/selective (e.g., OATP2 - liver; OATP-RP4 - heart and skeletal muscle, and OATP-RP5 - brain and testis), compound specificity is built into any specific substrate of these OATPs and into molecular carriers transported by these OATPs. An agent transported by the above OATPs of the present invention would thus be delivered to the tissues in which they are expressed and not to tissues lacking the above OATPs, thereby achieving tissue specific targeting.

The OATP nucleic acids of the present invention, or antisense nucleic acids, may be useful therapeutic or diagnostic agents. For such gene therapy, the nucleic acids may be incorporated into vectors and/or formulated as described below and in further detail in the art.

The present invention also provides a basis for diagnostic genetic screens for predicting response to drugs. At least one of the transporters disclosed and claimed herein is a transporter of a known drug (i.e., OATP2 transports pravastatin into hepatocytes). Other transporters disclosed herein may similarly transport additional drugs into tissues. Persons skilled in the art can: (1) screen the transporter genes for allelic variants (genotypes) in the general population by various sequencing methods; and (2) determine the association of these transporter genotypes in patients with response to the transported drug in clinical trials. Particular allelic variants may be more or less effective in transporting a drug, which would be related to drug efficacy. Thus, genotyping of the claimed transporters could form the basis of a clinical diagnostic test to predict a patient's response to drug therapy.

Persons skilled in the art can use the polypeptides and nucleic acids of this invention to prepare vectors, cells or cell lines, and antibodies. All of these are useful in assays for identification of OATP positive and negative modulators (i.e., agonists and/or antagonists) and OATP carriers. The term "positive modulator" as used herein

refers to an agent or compound that increases the rate or amount of transport of a compound into an organ, e.g., the liver, or an agent or compound that decreases the rate or amount of transport of a compound into an organ. The term "negative modulator" refers to a compound that is joined to a second compound to prevent the second compounds transport into or out of cells. The term "carrier" as used herein refers to an agent or compound that is transported by an OATP of the present invention and that is capable of being joined to or associated with another compound to chaperone that other compound into an organ, e.g., the liver. A carrier includes an agent that is used to transport a compound into an organ that is otherwise not transported into said organ, and includes an agent that increases the transport of a compound into an organ that is capable of being transported by an OATP.

One can administer OATP modulators and carriers to various mammalian species, such as monkeys, dogs, cats, mice, rats, humans, etc. By known methods, persons skilled in the pharmaceutical art can incorporate OATP modulators and carriers in a conventional systemic dosage form, such as a tablet, capsule, elixir or injectable formulation. The above dosage forms will also include any necessary physiologically acceptable carrier material, excipient, lubricant, buffer, antibacterial, bulking agent (such as mannitol), anti-oxidants (ascorbic acid or sodium bisulfite) or the like.

Process of preparation

In general

This specification describes the cloning and functional expression of full-length human cDNA clones of OATPs, preferably the nucleic acid sequence of OATP2 (SEQ ID NO:1), the amino acid sequence of OATP2 (SEQ ID NO:2), the nucleic acid sequence of OATP-RP2 (SEQ ID NO:3), the amino acid sequence of OATP-RP2 (SEQ ID NO:4), the nucleic acid sequence of OATP-RP3 (SEQ ID NO:5), the amino acid sequence of OATP-RP3 (SEQ ID NO:6), the nucleic acid sequence of OATP-RP4 (SEQ ID NO:7), the amino acid sequence of OATP-RP4 (SEQ ID NO:8), the nucleic acid sequence of OATP-RP5 (SEQ ID NO:9), the amino acid sequence of OATP-RP5 (SEQ ID NO:10), the nucleic acid sequence of OATP-RP1 (SEQ ID NO:11), and the amino acid sequence of OATP-RP1 (SEQ ID NO:12).

DNA clones comprising nucleotide sequences encoding the OATPs described above were deposited with the American Type Culture Collection ("ATCC") (10801 University Blvd., Manassas, VA 20110-2209) on April 20, 1999, and given the following ATCC Accession Numbers: 207209 (OATP-RP3), 207210 (OATP-RP4), 207211 (OATP-RP5), 207212 (OATP-RP2), 207213 (OATP2), and 207214 (OATP-RP1). The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

Nucleic acids

With the disclosed OATP gene sequences in hand, one skilled in the art can obtain OATP nucleic acids of this invention by known methods. Such methods include: (1) Southern and Northern blotting; (2) Western immunoblotting; (3) chemical synthesis; (4) synthesis by polymerase chain reaction (PCR) from primers; (5) expression cloning; and (6) subtractive cDNA cloning.

Preferred nucleic acid sequences of the present invention include the following (preferably the coding sequences as shown below):

OATP2 (SEQ ID NOS:1 and 2):

```
CGGACGCGTG GGCGGACGCG TGGGTCGCCC ACGCGTCCGA CTTGTTGCAG       50
TTGCTGTAGG ATTCTAAATC CAGGTGATTG TTTCAAACTG AGCATCAACA      100
ACAAAAACAT TTGTATGATA TCTATATTTC AATC ATG GAC CAA AAT CAA   149
                                       M   D   Q   N   Q

CAT TTG AAT AAA ACA GCA GAG GCA CAA CCT TCA GAG AAT AAG     191
 H   L   N   K   T   A   E   A   Q   P   S   E   N   K

AAA ACA AGA TAC TGC AAT GGA TTG AAG ATG TTC TTG GCA GCT     233
 K   T   R   Y   C   N   G   L   K   M   F   L   A   A

CTG TCA CTC AGC TTT ATT GCT AAG ACA CTA GGT GCA ATT ATT     275
 L   S   L   S   F   I   A   K   T   L   G   A   I   I
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ATG AAA AGT TCC ATC ATT CAT ATA GAA CGG AGA TTT GAG ATA 317
M K S S I I H I E R R F E I

TCC TCT TCT CTT GTT GGT TTT ATT GAC GGA AGC TTT GAA ATT 359
S S S L V G F I D G S F E I

GGA AAT TTG CTT GTG ATT GTA TTT GTG AGT TAC TTT GGA TCC 401
G N L L V I V F V S Y F G S

AAA CTA CAT AGA CCA AAG TTA ATT GGA ATC GGT TGT TTC ATT 443
K L H R P K L I G I G C F I

ATG GGA ATT GGA GGT GTT TTG ACT GCT TTG CCA CAT TTC TTC 485
M G I G G V L T A L P H F F

ATG GGA TAT TAC AGG TAT TCT AAA GAA ACT AAT ATC GAT TCA 527
M G Y Y R Y S K E T N I D S

TCA GAA AAT TCA ACA TCG ACC TTA TCC ACT TGT TTA ATT AAT 569
S E N S T S T L S T C L I N

CAA ATT TTA TCA CTC AAT AGA GCA TCA CCT GAG ATA GTG GGA 611
Q I L S L N R A S P E I V G

AAA GGT TGT TTA AAG GAA TCT GGG TCA TAC ATG TGG ATA TAT 653
K G C L K E S G S Y M W I Y

GTG TTC ATG GGT AAT ATG CTT CGT GGA ATA GGG GAG ACT CCC 695
V F M G N M L R G I G E T P

ATA GTA CCA TTG GGG CTT TCT TAC ATT GAT GAT TTC GCT AAA 737
I V P L G L S Y I D D F A K

GAA GGA CAT TCT TCT TTG TAT TTA GGT ATA TTG AAT GCA ATA 779
E G H S S L Y L G I L N A I

GCA ATG ATT GGT CCA ATC ATT GGC TTT ACC CTG GGA TCT CTG 821
A M I G P I I G F T L G S L

TTT TCT AAA ATG TAC GTG GAT ATT GGA TAT GTA GAT CTA AGC 863
F S K M Y V D I G Y V D L S

ACT ATC AGG ATA ACT CCT ACT GAT TCT CGA TGG GTT GGA GCT 905
T I R I T P T D S R W V G A

TGG TGG CTT AAT TTC CTT GTG TCT GGA CTA TTC TCC ATT ATT 947
W W L N F L V S G L F S I I

TCT TCC ATA CCA TTC TTT TTC TTG CCC CAA ACT CCA AAT AAA 989
S S I P F F F L P Q T P N K

CCA CAA AAA GAA AGA AAA GCT TCA CTG TCT TTG CAT GTG CTG 1031
P Q K E R K A S L S L H V L

GAA ACA AAT GAT GAA AAG GAT CAA ACA GCT AAT TTG ACC AAT 1073
E T N D E K D Q T A N L T N

CAA GGA AAA AAT ATT ACC AAA AAT GTG ACT GGT TTT TTC CAG 1115
Q G K N I T K N V T G F F Q

TCT TTT AAA AGC ATC CTT ACT AAT CCC CTG TAT GTT ATG TTT 1157
S F K S I L T N P L Y V M F

GTG CTT TTG ACG TTG TTA CAA GTA AGC AGC TAT ATT GGT GCT 1199
V L L T L L Q V S S Y I G A

TTT ACT TAT GTC TTC AAA TAC GTA GAG CAA CAG TAT GGT CAG 1241
F T Y V F K Y V E Q Q Y G Q

CCT TCA TCT AAG GCT AAC ATC TTA TTG GGA GTC ATA ACC ATA 1283
P S S K A N I L L G V I T I

CCT ATT TTT GCA AGT GGA ATG TTT TTA GGA GGA TAT ATC ATT 1325
P I F A S G M F L G G Y I I

AAA AAA TTC AAA CTG AAC ACC GTT GGA ATT GCC AAA TTC TCA 1367
K K F K L N T V G I A K F S

TGT TTT ACT GCT GTG ATG TCA TTG TCC TTT TAC CTA TTA TAT 1409
C F T A V M S L S F Y L L Y

TTT TTC ATA CTC TGT GAA AAC AAA TCA GTT GCC GGA CTA ACC 1451
F F I L C E N K S V A G L T

ATG ACC TAT GAT GGA AAT AAT CCA GTG ACA TCT CAT AGA GAT 1493
M T Y D G N N P V T S H R D

GTA CCA CTT TCT TAT TGC AAC TCA GAC TGC AAT TGT GAT GAA 1535
V P L S Y C N S D C N C D E

AGT CAA TGG GAA CCA GTC TGT GGA AAC AAT GGA ATA ACT TAC 1577
S Q W E P V C G N N G I T Y

ATC TCA CCC TGT CTA GCA GGT TGC AAA TCT TCA AGT GGC AAT 1619
I S P C L A G C K S S S G N

AAA AAG CCT ATA GTG TTT TAC AAC TGC AGT TGT TTG GAA GTA 1661
K K P I V F Y N C S C L E V

ACT GGT CTC CAG AAC AGA AAT TAC TCA GCC CAT TTG GGT GAA 1703
T G L Q N R N Y S A H L G E

TGC CCA AGA GAT GAT GCT TGT ACA AGG AAA TTT TAC TTT TTT 1745
C P R D D A C T R K F Y F F

GTT GCA ATA CAA GTC TTG AAT TTA TTT TTC TCT GCA CTT GGA 1787
V A I Q V L N L F F S A L G

GGC ACC TCA CAT GTC ATG CTG ATT GTT AAA ATT GTT CAA CCT 1829
G T S H V M L I V K I V Q P

GAA TTG AAA TCA CTT GCA CTG GGT TTC CAC TCA ATG GTT ATA 1871

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 E   L   K   S   L   A   L   G   R   H   S   M   V   I

CGA GCA CTA GGA GGA ATT CTA GCT CCA ATA TAT TTT GGG GCT     1913
 R   A   L   G   G   I   L   A   P   I   Y   F   G   A

CTG ATT GAT ACA ACG TGT ATA AAG TGG TCC ACC AAC AAC TGT     1955
 L   I   D   T   T   C   I   K   W   S   T   N   N   C

GGC ACA CGT GGG TCA TGT AGG ACA TAT AAT TCC ACA TCA TTT     1997
 G   T   R   G   S   C   R   T   Y   N   S   T   S   F

TCA AGG GTC TAC TTG GGC TTG TCT TCA ATG TTA AGA GTC TCA     2039
 S   R   V   Y   L   G   L   S   S   M   L   R   V   S

TCA CTT GTT TTA TAT ATT ATA TTA ATT TAT GCC ATG AAG AAA     2081
 S   L   V   L   Y   I   I   L   I   Y   A   M   K   K

AAA TAT CAA GAG AAA GAT ATC AAT GCA TCA GAA AAT GGA AGT     2123
 K   Y   Q   E   K   D   I   N   A   S   E   N   G   S

GTC ATG GAT GAA GCA AAC TTA GAA TCC TTA AAT AAA AAT AAA     2165
 V   M   D   E   A   N   L   E   S   L   N   K   N   K

CAT TTT GTC CCT TCT GCT GGG GCA GAT AGT GAA ACA CAT TGT     2207
 H   F   V   P   S   A   G   A   D   S   E   T   H   C

TAA GGGGAGAAAA AAAGCCACTT CTGCTTCTGT GTTTCCAAAC AGCATTGCAT 2260
 *

TGATTCAGTA AGATGTTATT TTTGAGGAGT TCCTGGTCCT TTCACTAAGA     2310
ATTTCCACAT CTTTTATGGT GGAAGTATAA ATAAGCCTAT GAACTTATAA     2360
TAAAACAAAC TGTAGGTAGA AAAAATGAGA GTACTCATTG TTACATTATA     2410
GCTACATATT TGTGGTTAAG GTTAGACTAT ATGATCCATA CAAATTAAAG     2460
TGAGAGACAT GGTTACTGTG TAATAAAAGA AAAAATACTT GTTCAGGTAA     2510
TTCTAATTCT TAATAAAACA AATGAGTATC ATACAGGTAG AGGTTAAAAA     2560
GGAGGAGCTA GATTCATATC CTAAGTAAAG AGAAATGCCT AGTGTCTATT     2610
TTATTAAACA AACAAACACA GAGTTTGAAC TATAATACTA AGGCCTGAAG     2660
TCTAGCTTGG ATATATGCTA CAATAATATC TGTTACTCAC ATAAAATTAT     2710
ATATTTCACA GACTTTATCA ATGTATAATT AACAATTATC TTGTTTAAGT     2760
AAATTTAGAA TACATTTAAG TATTGTGGAA GAAATAAAGA CATTCCAATA     2810
TTTGCAAAAA AAAAAAAAAA                                      2830
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OATP-RP2 (SEQ ID NOS:3 and 4):

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CCCGGGTCGA CCCACGCGTC CGGGATAAAG TACTCCCAGG AAGGCTTTGA       50
GCCTTGGCAG AAGAGGCTGG GATTGAAGCT TCAGGGAGAG CCAGAGGTGA      100
GGCTGGAGTG GGAGATCACC TGAGGCAGGG CCAGCGGGTG AGGTACCCCA      150
GGTACCAGAC AAGGAAACCA AAGCCACA ATG GGC ACA GAA AAC ACA CCT  199
                                M   G   T   E   N   T   P

GGA GGC AAA GCC AGC CCA GAC CCT CAG GAC GTG CGG CCA AGT     241
 G   G   K   A   S   P   D   P   Q   D   V   R   P   S

GTG TTC CAT AAC ATC AAG CTG TTC GTT CTG TGC CAC AGC CTG     283
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SUBSTITUTE SHEET (RULE 26)

V F H N I K L F V L C H S L

CTG CAG CTG GCG CAG CTC ATG ATC TCC GGC TAC CTA AAG AGC 325
L Q L A Q L M I S G Y L K S

TCC ATC TCC ACA GTG GAG AAG CGC TTC GGC CTC TCC AGC CAG 367
S I S T V E K R F G L S S Q

ACG TCG GGG CTG CTG GCC TCC TTC AAC GAG GTG GGG AAC ACA 409
T S G L L A S F N E V G N T

GCC TTG ATT GTG TTT GTG AGC TAT TTT GGC AGC CGG GTG CAC 451
A L I V F V S Y F G S R V H

CGA CCC CGA ATG ATT GGC TAT GGG GCT ATC CTT GTG GCC CTG 493
R P R M I G Y G A I L V A L

GCG GGC CTG CTC ATG ACT CTC CCG CAC TTC ATC TCG GAG CCA 535
A G L L M T L P H F I S E P

TAC CGC TAC GAC AAC ACC AGC CCT GAG GAT ATG CCA CAG GAC 577
Y R Y D N T S P E D M P Q D

TTC AAG GCT TCC CTG TGC CTG CCC ACA ACC TCG GCC CCA GCC 619
F K A S L C L P T T S A P A

TCG GCC CCC TCC AAT GGC AAC TGC TCA AGC TAC ACA GAA ACC 661
S A P S N G N C S S Y T E T

CAG CAT CTG AGT GTG GTG GGG ATC ATG TTC GTG GCA CAG ACC 703
Q H L S V V G I M F V A Q T

CTG CTG GGC GTG GGC GGG GTG CCC ATT CAG CCC TTT GGC ATC 745
L L G V G G V P I Q P F G I

TCC TAC ATC GTT GAC TTT GCC CAC AAC AGT AAC TCG CCC CTC 787
S Y I V D F A H N S N S P L

TAC CTC GGG ATC CTG TTT GCA GTG ACC ATG ATG GGG CCA GGC 829
Y L G I L F A V T M M G P G

CTG GCC TTT GGG CTG GGC AGC CTC ATG CTG CGC CTT TAT GTG 871
L A F G L G S L M L R L Y V

GAC ATT AAC CAG ATG CCA GAA GGT GGT ATC AGC CTG ACC ATA 913
D I N Q M P E G G I S L T I

AAG GAC CCC CGA TGG GTG GGT GCC TGG TGG CTG GGT TTC CTC 955
K D P R W V G A W W L G F L

ATC GCT GCC GGT GCA GTG GCC CTG GCT GCC ATC CCC TAC TTC 997
I A A G A V A L A A I P Y F

TTC TTC CCC AAG GAA ATG CCC AAG GAA AAA CGT GAG CTT CAG 1039
F F P K E M P K E K R E L Q

TTT CGG CGA AAG GTC TTA GCA GTC ACA GAC TCA CCT GCC AGG 1081
F R R K V L A V T D S P A R

AAG GGC AAG GAC TCT CCC TCT AAG CAG AGC CCT GGG GAG TCC 1123
K G K D S P S K Q S P G E S

ACG AAG AAG CAG GAT GGC CTA GTC CAG ATT GCA CCA AAC CTG 1165
T K K Q D G L V Q I A P N L

ACT GTG ATC CAG TTC ATT AAA GTC TTC CCC AGG GTG CTG CTG 1207
T V I Q F I K V F P R V L L

CAG ACC CTA CGC CAC CCC ATC TTC CTG CTG GTG GTC CTG TCC 1249
Q T L R H P I F L L V V L S

CAG GTA TGC TTG TCA TCC ATG GCT GCG GGC ATG GCC ACC TTC 1291
Q V C L S S M A A G M A T F

CTG CCC AAG TTC CTG GAG CGC CAG TTT TCC ATC ACA GCC TCC 1333
L P K F L E R Q F S I T A S

TAC GCC AAC CTG CTC ATC GGC TGC CTC TCC TTC CCT TCG GTC 1375
Y A N L L I G C L S F P S V

ATC GTG GGC ATC GTG GTG GGT GGC GTC CTG GTC AAG CGG CTC 1417
I V G I V V G G V L V K R L

CAC CTG GGC CCT GTG GGA TGC GGT GCC CTT TGC CTG CTG GGG 1459
H L G P V G C G A L C L L G

ATG CTG CTG TGC CTC TTC TTC AGC CTG CCG CTC TTC TTT ATC 1501
M L L C L F F S L P L F F I

GGC TGC TCC AGC CAC CAG ATT GCG GGC ATC ACA CAC CAG ACC 1543
G C S S H Q I A G I T H Q T

AGT GCC CAC CCT GGG CTG GAG CTG TCT CCA AGC TGC ATG GAG 1585
S A H P G L E L S P S C M E

GCC TGC TCC TGC CCA TTG GAC GGC TTT AAC CCT GTC TGC GAC 1627
A C S C P L D G F N P V C D

CCC AGC ACT CGT GTG GAA TAC ATC ACA CCC TGC CAC GCA GGC 1669
P S T R V E Y I T P C H A G

TGC TCA AGC TGG GTG GTC CAG GAT GCT CTG GAC AAC AGC CAG 1711
C S S W V V Q D A L D N S Q

GTT TTC TAC ACC AAC TGC AGC TGC GTG GTG GAG GGC AAC CCC 1753
V F Y T N C S C V V E G N P

GTG CTG GCA GGA TCC TGC GAC TCA ACG TGC AGC CAT CTG GTG 1795
V L A G S C D S T C S H L V

GTG CCC TTC CTG CTC CTG GTC AGC CTG GGC TCG GCC CTG GCC 1837
V P F L L L V S L G S A L A

TGT CTC ACC CAC ACA CCC TCC TTC ATG CTC ATC CTA AGA GGA 1879

C L T H T P S F M L I L R G

GTG AAG AAA GAA GAC AAG ACT TTG GCT GTG GGC ATC CAG TTC 1921

V K K E D K T L A V G I Q F

ATG TTC CTG AGG ATT TTG GCC TGG ATG CCC AGC CCC GTG ATC 1963

M F L R I L A W M P S P V I

CAC GGC AGC GCC ATC GAC ACC ACC TGT GTG CAC TGG GCC CTG 2005

H G S A I D T T C V H W A L

AGC TGT GGG CGT CGA GCT GTC TGT CGC TAC TAC AAT AAT GAC 2047

S C G R R A V C R Y Y N N D

CTG CTC CGA AAC CGG TTC ATC GGC CTC CAG TTC TTC TTC AAA 2089

L L R N R F I G L Q F F F K

ACA GGT TCT GTG ATC TGC TTC GCC TTA GTT TTG GCT GTC CTG 2131

T G S V I C F A L V L A V L

AGG CAG CAG GAC AAA GAG GCA AGG ACC AAA GAG AGC AGA TCC 2173

R Q Q D K E A R T K E S R S

AGC CCT GCC GTA GAG CAG CAA TTG CTA GTG TCG GGG CCA GGG 2215

S P A V E Q Q L L V S G P G

AAG AAG CCA GAG GAT TCC CGA GTG TGA GCTGTCTTGG GGCCCCACCT 2262

K K P E D S R V *

GGCCAAGAGT AGCAGCCACA GCAGTACCTC CTCTGAGTCC TTTGCCCAAG 2312

ATTGGGTGTC AAGAGCCCTG TGTTCCATTC TGGCTCCTCC ACTAAATTGC 2362

TGTGTGACTT CAGGCAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 2412

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 2442

OATP-RP3 (SEQ ID NOS:5 and 6):

CC CACGCGTCCG 12

GCGAGGAGCT GTGCCTTCCA CCTCTCCAGC CCCGGCAGGA CGGGGGCGGC 62

CGCCGCGAAC CCGGGGCGGG GACAGCACGC AGCCTCGAGG CGCGCACCCC 112

CGCCCGGCAG CGGCCCCGAC ACCCGGGGCG AGCGGGAAAG CGGCAGCGGC 162

GGCGGCGGCG GCGGCGGCGG GGGAAGG ATG CAG GGG AAG AAG CCG GGC 210

M Q G K K P G

GGT TCG TCG GGC GGC GGC CGG AGC GGC GAG CTG CAG GGG GAC 252

G S S G G G R S G E L Q G D

GAG GCG CAG AGG AAC AAG AAA AAG AAA AAG AAG GTG TCC TGC 294

E A Q R N K K K K K K V S C

TTT TCC AAC ATC AAG ATC TTC CTG GTG TCC GAG TGC GCC CTG 336

F S N I K I F L V S E C A L

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ATG CTG GCG CAG GGC ACG GTG GGC GCC TAC CTG GTG AGC GTC   378
 M   L   A   Q   G   T   V   G   A   Y   L   V   S   V

CTG ACC ACC CTG GAG CGT AGG TTC AAC CTG CAG AGC GCT GAC   420
 L   T   T   L   E   R   R   F   N   L   Q   S   A   D

GTG GGT GTG ATC GCT AGC AGC TTC GAG ATC GGG AAC CTG GCG   462
 V   G   V   I   A   S   S   F   E   I   G   N   L   A

CTC ATC CTC TTC GTG AGC TAC TTC GGG GCA CGC GGG CAC CGG   504
 L   I   L   F   V   S   Y   F   G   A   R   G   H   R

CCG CGC CTG ATC GGC TGC GGC GGC ATC GTC ATG GCG CTG GGC   546
 P   R   L   I   G   C   G   G   I   V   M   A   L   G

GCG CTG CTG TCG GCG CTG CCC GAG TTC CTG ACC CAC CAG TAC   588
 A   L   L   S   A   L   P   E   F   L   T   H   Q   Y

AAG TAC GAG GCG GGC GAG ATC CGC TGG GGC GCC GAG GGC CGC   630
 K   Y   E   A   G   E   I   R   W   G   A   E   G   R

GAC GTC TGC GCA GCC AAC GGC TCG GGC GGC GAC GAG GGC CCC   672
 D   V   C   A   A   N   G   S   G   G   D   E   G   P

GAC CCC GAC CTC ATC TGC CGC AAC CGG ACG GCT ACC AAC ATG   714
 D   P   D   L   I   C   R   N   R   T   A   T   N   M

ATG TAC TTG CTG CTC ATT GGG GCC CAG GTG CTC CTG GGC ATC   756
 M   Y   L   L   L   I   G   A   Q   V   L   L   G   I

GGT GCT ACC CCT GTG CAG CCC CTG GGC GTC TCC TAC ATC GAC   798
 G   A   T   P   V   Q   P   L   G   V   S   Y   I   D

GAC CAC GTG CGG AGG AAG GAC TCC TCG CTC TAT ATA GGA ATC   840
 D   H   V   R   R   K   D   S   S   L   Y   I   G   I

CTG TTC ACG ATG CTG GTA TTT GGA CCA GCC TGC GGG TTT ATC   882
 L   F   T   M   L   V   F   G   P   A   C   G   F   I

CTG GGC TCT TTC TGT ACC AAA ATC TAC GTG GAT GCG GTC TTC   924
 L   G   S   F   C   T   K   I   Y   V   D   A   V   F

ATT GAC ACA AGT AAC CTG GAC ATC ACT CCG GAC GAC CCC CGC   966
 I   D   T   S   N   L   D   I   T   P   D   D   P   R

TGG ATC GGA GCC TGG TGG GGT GGC TTT CTG CTC TGC GGT GCC   1008
 W   I   G   A   W   W   G   G   F   L   L   C   G   A

TTA CTC TTC TTC TCT TCC CTC TTG ATG TTT GGG TTT CCA CAG   1050
 L   L   F   F   S   S   L   L   M   F   G   F   P   Q

TCC CTG CCC CCG CAC TCA GAC CCC GCC ATG GAA AGC GAG CAG   1092
 S   L   P   P   H   S   D   P   A   M   E   S   E   Q

GCC ATG CTC TCC GAA AGA GAA TAC GAG AGA CCC AAG CCC AGC   1134
 A   M   L   S   E   R   E   Y   E   R   P   K   P   S
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AAC GGG GTC CTG AGG CAC CCC CTG GAG CCA GAC AGC AGT GCC     1176
 N   G   V   L   R   H   P   L   E   P   D   S   S   A

TCC TGT TTC CAG CAG CTG AGA GTG ATC CCG AAG GTC ACC AAG     1218
 S   C   F   Q   Q   L   R   V   I   P   K   V   T   K

CAC CTG CTC TCA AAC CCT GTG TTC ACC TGC ATC ATC CTG GCC     1260
 H   L   L   S   N   P   V   F   T   C   I   I   L   A

GCC TGC ATG GAG ATT GCA GTG GTG GCT GGC TTC GCT GCC TTT     1302
 A   C   M   E   I   A   V   V   A   G   F   A   A   F

TTG GGG AAG TAC CTG GAG CAG CAG TTT AAC CTC ACC ACC TCT     1344
 L   G   K   Y   L   E   Q   Q   F   N   L   T   T   S

TCT GCC AAC CAG CTG CTT GGG ATG ACT GCG ATC CCG TGT GCT     1386
 S   A   N   Q   L   L   G   M   T   A   I   P   C   A

TGT CTG GGT ATC TTC CTG GGA GGT CTT TTG GTG AAG AAG CTC     1428
 C   L   G   I   F   L   G   G   L   L   V   K   K   L

AGC CTG TCT GCC CTG GGG GCC ATT CGG ATG GCC ATG CTC GTC     1470
 S   L   S   A   L   G   A   I   R   M   A   M   L   V

AAC CTG GTG TCC ACT GCT TGC TAC GTC TCC TTC CTC TTC CTG     1512
 N   L   V   S   T   A   C   Y   V   S   F   L   F   L

GGC TGC GAC ACT GGC CCT GTG GCT GGG GTT ACT GTT CCC TAT     1554
 G   C   D   T   G   P   V   A   G   V   T   V   P   Y

GGA AAC AGC ACA GCA CCT GGC TCA GCC CTG GAC CCC TAC TCG     1596
 G   N   S   T   A   P   G   S   A   L   D   P   Y   S

CCC TGC AAT AAT AAC TGT GAA TGC CAA ACC GAT TCC TTC ACT     1638
 P   C   N   N   N   C   E   C   Q   T   D   S   F   T

CCA GTG TGT GGG GCA GAT GGC ATC ACC TAC CTG TCT GCC TGC     1680
 P   V   C   G   A   D   G   I   T   Y   L   S   A   C

TTT GCT GGC TGC AAC AGC ACG AAT CTC ACG GGC TGT GCG TGC     1722
 F   A   G   C   N   S   T   N   L   T   G   C   A   C

CTC ACC ACC GTC CCT GCT GAG AAC GCA ACC GTG GTT CCT GGA     1764
 L   T   T   V   P   A   E   N   A   T   V   V   P   G

AAA TGC CCC AGT CCT GGG TGC CAA GAG GCC TTC CTC ACT TTC     1806
 K   C   P   S   P   G   C   Q   E   A   F   L   T   F

CTC TGT GTG ATG TGT ATC TGC AGC CTG ATC GGT GCC ATG GCA     1848
 L   C   V   M   C   I   C   S   L   I   G   A   M   A

CAG ACA CCC TCA GTC ATC ATC CTC ATC AGG ACA GTC AGC CCT     1890
 Q   T   P   S   V   I   I   L   I   R   T   V   S   P

GAA CTC AAG TCT TAC GCT TTG GGA GTT CTT TTT CTC CTC CTT     1932
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E L K S Y A L G V L F L L L

CGT TTG TTG GGC TTC ATC CCT CCA CCC CTC ATC TTC GGG GCT 1974
R L L G F I P P P L I F G A

GGC ATC GAC TCC ACC TGC CTG TTC TGG AGC ACG TTC TGT GGG 2016
G I D S T C L F W S T F C G

GAG CAA GGC GCC TGC GTC CTC TAC GAC AAT GTG GTC TAC CGA 2058
E Q G A C V L Y D N V V Y R

TAC CTG TAT GTC AGC ATC GCC ATC GCG CTC AAA TCC TTC GCC 2100
Y L Y V S I A I A L K S F A

TTC ATC CTG TAC ACC ACC ACG TGG CAG TGC CTG AGG AAA AAC 2142
F I L Y T T T W Q C L R K N

TAT AAA CGC TAC ATC AAA AAC CAC GAG GGC GGG CTG AGC ACC 2184
Y K R Y I K N H E G G L S T

AGT GAG TTC TTT GCC TCT ACT CTG ACC CTA GAC AAC CTG GGG 2226
S E F F A S T L T L D N L G

AGG GAC CCT GTG CCC GCA AAC CAG ACA CAT AGG ACA AAG TTT 2268
R D P V P A N Q T H R T K F

ATC TAT AAC CTG GAA GAC CAT GAG TGG TGT GAA AAC ATG GAG 2310
I Y N L E D H E W C E N M E

TCC GTT TTA TAG TGACTAAAGG AGGGCTGAAC TCTGTATTAG TAATCCAAGG 2362
S V L *

GTCATTTTTT TCTTAAAAAA AGAAAAAAAG GTTCCAAAAA AAACCAAAAC 2412
TCAGTACACA CACACAGGCA CAGATGCACA CACACGCAGA CAGACACACC 2462
GACTTTGTCC TTTTTCTCAG CATCAGAGCC AGACAGGATT CAGAATAAGG 2512
AGAGAATGAC ATCGTGCGGC AGGGTCCTGG AGGCCACTCG CGCGGCTGGG 2562
CCACAGAGTC TACTTTGAAG GCACCTCATG GTTTTCAGGA TGCTGACAGC 2612
TGCAAGCAAC AGGCACTGCC AAATTCAGGG AACAGTGGTG GCCAGCTTGG 2662
AGGATGGACA TTTCTGGATA CACATACACA TACAAAACAG AAAACATTTT 2712
TTAAAAGAAG TTTCCTAAAA TAAAAAAAAT AAAAAAAAAA AAAAA 2757

OATP-RP4 (SEQ ID NOS:7 and 8) (Nucleotide 713, designated Y, can be either a C (in which case the encoded amino acid X is Leu) or a T (in which case the encoded amino acid X is Phe); Nucleotide 2397, designated K, can be either a G (in which case the encoded amino acid X is Gly) or a T (in which case the encoded amino acid X is Val)):

CTGATTTCTC TTCGGCTGGA CGGAGGCTGC CTCCTCACGC GGCTCCCAAC 50
TATTCCCGTA GCTCAGTGCC CCCCTCCCGC CGCTCTACTC AGCCAGGCAG 100
ACAGACTGAC AGACTCGCTA GTCGGCAGCT TCACTCCCGA GGGTGCCGCG 150
AGCCCAGGCG GCGAACACCC GGTACCCCTG GCGCAGCGAG GTGGGATGCT 200

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GTACGGACAG CAGCGCTAAG TGCCCCCCCA CCCCCGGCGC AGGGTGCACT   250
CGCTCCTGGC CGCGGGCCCA GCGGCGGCGG CGGCGGCGGC GGCGGAGGGG   300
ATGAGCCCGG GACGCGCGAG GCGCCTGCCT CAAGCTACCG CCCGGAGAGG   350
GACGCCGAGT AGGGCTCATC GCAGTACCGC GCGGACCCCT GCCCCCTGTG   400
GCACGCGGCT GCGGAGCCTT GAAGCCGTGT CTGTGATCAG GATGCACTGG   450
GCGCCTCGCA GCTGGTGAGG ATGCCCTGCT GCGCGGCCCT GCGCCCCCAG   500
CCCCAGTCCC AGGTGGGCAA GACTGACTGG GCCCGGCTTC GGCCCCTCGT   550
GCCGGTGGAT GAAACGTGCC GGAGTGCTTG GGTGCCATCA GCTATCAAAT   600
CTGAATTCTA AGCGCC ATG GAC GAA GGC ACT GGA CTG CAG CCC GGG   646
                   M   D   E   G   T   G   L   Q   P   G

GCG GGA GAG CAG CTG GAG GCG CCG GCC ACT GCA GAA GCT GTC   688
 A   G   E   Q   L   E   A   P   A   T   A   E   A   V

CAA GAG AGG TGC GAG CCG GAG ACC YTC AGG TCT AAG AGT TTA   730
 Q   E   R   C   E   P   E   T   X   R   S   K   S   L

CCG GTC CTC AGC AGC GCC TCC TGC CGG CCA AGC CTC AGT CCC   772
 P   V   L   S   S   A   S   C   R   P   S   L   S   P

ACT AGT GGA GAC GCC AAC CCG GCC TTT GGC TGT GTG GAT TCT   814
 T   S   G   D   A   N   P   A   F   G   C   V   D   S

TCG GGC CAC CAG GAG TTG AAG CAA GGC CCG AAC CCG TTG GCC   856
 S   G   H   Q   E   L   K   Q   G   P   N   P   L   A

CCC AGT CCC TCT GCC CCG TCC ACT TCG GCG GGG CTC GGG GAC   898
 P   S   P   S   A   P   S   T   S   A   G   L   G   D

TGT AAC CAC AGG GTG GAC CTC AGC AAA ACC TTC TCG GTG TCC   940
 C   N   H   R   V   D   L   S   K   T   F   S   V   S

TCC GCC TTG GCC ATG CTC CAG GAG AGA AGG TGC CTC TAC GTG   982
 S   A   L   A   M   L   Q   E   R   R   C   L   Y   V

GTC CTC ACG GAT TCC CGT TGC TTC CTG GTG TGC ATG TGC TTT   1024
 V   L   T   D   S   R   C   F   L   V   C   M   C   F

CTG ACC TTC ATC CAG GCG TTA ATG GTC TCT GGG TAC CTG AGC   1066
 L   T   F   I   Q   A   L   M   V   S   G   Y   L   S

AGC GTA ATT ACC ACC ATT GAA AGG CGC TAC AGT CTG AAG AGT   1108
 S   V   I   T   T   I   E   R   R   Y   S   L   K   S

TCC GAG TCG GGG CTG CTG GTC AGC TGC TTT GAC ATC GGG AAC   1150
 S   E   S   G   L   L   V   S   C   F   D   I   G   N

CTG GTG GTG GTG GTG TTC GTC AGC TAC TTC GGC GGC CGG GGT   1192
 L   V   V   V   V   F   V   S   Y   F   G   G   R   G

CGG CGG CCC CTG TGG CTG GCC GTG GGT GGA CTC CTC ATC GCC   1234
 R   R   P   L   W   L   A   V   G   G   L   L   I   A

TTC GGG GCA GCC CTC TTC GCC TTA CCT CAC TTC ATC TCG CCC   1276
 F   G   A   A   L   F   A   L   P   H   F   I   S   P
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CCC TAC CAG ATC CAA GAG TTG AAC GCC TCG GCC CCC AAC GAC 1318
P Y Q I Q E L N A S A P N D

GGC CTG TGT CAG GGT GGC AAC TCC ACC GCC ACT TTG GAG CCT 1360
G L C Q G G N S T A T L E P

CCG GCC TGT CCG AAG GAC TCG GGA GGA AAT AAT CAC TGG GTC 1402
P A C P K D S G G N N H W V

TAC CTG GCT TTA TTC ATT TGC GCG CAG ATT CTC ATT GGA ATG 1444
Y L A L F I C A Q I L I G M

GGC TCC ACA CCT ATT TAT ACC CTG GGA CCA ACC TAC TTA GAT 1486
G S T P I Y T L G P T Y L D

GAC AAT GTC AAG AAA GAA AAC TCC TCC TTG TAC CTA GCC ATC 1528
D N V K K E N S S L Y L A I

ATG TAT GTC ATG GGA GCA CTT GGC CCT GCA GTG GGA TAT TTA 1570
M Y V M G A L G P A V G Y L

TTA GGT GGA CTT CTT ATT GGT TTT TAT GTT GAT CCC AGA AAT 1612
L G G L L I G F Y V D P R N

CCT GTT CAC CTT GAC CAG AAT GAC CCT CGT TTC ATT GGA AAC 1654
P V H L D Q N D P R F I G N

TGG TGG AGT GGA TTC CTC CTT TGT GCC ATT GCA ATG TTT CTT 1696
W W S G F L L C A I A M F L

GTG ATA TTC CCA ATG TTT ACT TTC CCA AAA AAG CTT CCA CCT 1738
V I F P M F T F P K K L P P

CGA CAC AAG AAA AAG AAA AAG AAA AAA TTT TCT GTT GAT GCT 1780
R H K K K K K K K F S V D A

GTT AGT GAT GAC GAT GTT CTG AAG GAG AAA TCA AAC AAC AGT 1822
V S D D D V L K E K S N N S

GAA CAA GCG GAC AAA AAA GTT TCT TCG ATG GGA TTT GGA AAG 1864
E Q A D K K V S S M G F G K

GAT GTC AGA GAC CTA CCA AGA GCA GCT GTC AGG ATC TTA AGC 1906
D V R D L P R A A V R I L S

AAC ATG ACA TTC CTT TTT GTG AGT TTG TCA TAC ACA GCT GAG 1948
N M T F L F V S L S Y T A E

AGT GCC ATT GTA ACT GCT TTC ATT ACC TTC ATT CCC AAG TTC 1990
S A I V T A F I T F I P K F

ATC GAG TCA CAG TTT GGT ATC CCA GCC TCC AAT GCC AGC ATC 2032
I E S Q F G I P A S N A S I

TAC ACT GGG GTT ATT ATC GTC CCC AGT GCT GGT GTT GGT ATT 2074
Y T G V I I V P S A G V G I

GTC CTC GGA GGC TAC ATT ATA AAA AAA TTG AAA CTT GGT GCC 2116
V L G G Y I I K K L K L G A

AGA GAA TCT GCA AAA CTA GCA ATG ATC TGC AGT GGT GTG TCT 2158
R E S A K L A M I C S G V S

TTA CTA TGT TTT TCA ACC CTA TTT ATT GTT GGA TGT GAA AGC 2200
L L C F S T L F I V G C E S

ATT AAT CTA GGG GGC ATA AAC ATC CCT TAT ACA ACA GGA CCT 2242
I N L G G I N I P Y T T G P

TCT CTC ACC ATG CCC CAT AGG AAT CTG ACA GGA AGC TGC AAC 2284
S L T M P H R N L T G S C N

GTT AAT TGT GGT TGT AAA ATA CAC GAG TAT GAG CCA GTC TGT 2326
V N C G C K I H E Y E P V C

GGA TCA GAT GGA ATT ACA TAC TTT AAC CCT TGT CTG GCT GGC 2368
G S D G I T Y F N P C L A G

TGT GTT AAT AGT GGT AAT CTT AGC ACT GKG ATA CGG AAT TAT 2410
C V N S G N L S T X I R N Y

ACA GAA TGC ACC TGT GTC CAA AGT CGC CAA GTG ATC ACT CCA 2452
T E C T C V Q S R Q V I T P

CCC ACC GTG GGA CAG CGA AGT CAG CTC CGT GTG GTT ATT GTC 2494
P T V G Q R S Q L R V V I V

AAG ACT TAT CTC AAT GAG AAC GGC TAT GCT GTG TCT GGG AAA 2536
K T Y L N E N G Y A V S G K

TGT AAA CGG ACC TGC AAT ACT CTT ATC CCA TTC TTA GTT TTT 2578
C K R T C N T L I P F L V F

CTT TTC ATA GTC ACC TTC ATC ACA GCA TGT GCC CAA CCA TCA 2620
L F I V T F I T A C A Q P S

GCT ATC ATA GTA ACA CTC AGG TCC GTA GAA GAT GAG GAG AGA 2662
A I I V T L R S V E D E E R

CCT TTT GCA CTG GGA ATG CAG TTT GTT TTG TTG CGA ACA CTT 2704
P F A L G M Q F V L L R T L

GCA TAC ATT CCT ACT CCA ATC TAC TTT GGA GCA GTC ATT GAC 2746
A Y I P T P I Y F G A V I D

ACC ACC TGC ATG CTC TGG CAA CAG GAA TGT GGT GTG CAG GGT 2788
T T C M L W Q Q E C G V Q G

TCT TGC TGG GAG TAC AAC GTG ACG TCG TTT CGT TTT GTG TAT 2830
S C W E Y N V T S F R F V Y

TTT GGT TTG GCT GCC GGC CTC AAA TTC GTT GGG TTT ATT TTT 2872

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 F   G   L   A   A   G   L   K   F   V   G   F   I   F

ATT TTT CTG GCC TGG TAC TCC ATA AAA TAC AAG GAG GAT GGA   2914
 I   F   L   A   W   Y   S   I   K   Y   K   E   D   G

CTG CAG AGG CGG AGG CAG AGA GAA TTT CCC CTG AGC ACC GTG   2956
 L   Q   R   R   R   Q   R   E   F   P   L   S   T   V

AGT GAG AGA GTG GGA CAC CCC GAC AAT GCC CGG ACT AGA TCT   2998
 S   E   R   V   G   H   P   D   N   A   R   T   R   S

TGC CCA GCT TTC AGC ACC CAG GGA GAA TTC CAC GAA GAG ACT   3040
 C   P   A   F   S   T   Q   G   E   F   H   E   E   T

GGC CTG CAA AAA GGG ATC CAG TGC GCA GCA CAG ACC TAC CCG   3082
 G   L   Q   K   G   I   Q   C   A   A   Q   T   Y   P

GGG CCC TTC CCA GAA GCA ATA AGT TCC TCT GCG GAC CCG GGG   3124
 G   P   F   P   E   A   I   S   S   S   A   D   P   G

CTG GAA GAG AGC CCC GCT GCC TTG GAG CCG CCC TCC TGA       3163
 L   E   E   S   P   A   A   L   E   P   P   S   *

AGCTTGAAAA TGGAAGAATT TAGTTTTGTT GGTTGAATTG AAAATGGCGA   3213
CTTGAGAAAC AACTGTGCCT TCTTTTCTTT CTTTCTTTTT TTTAACCTCT   3263
ACAGACACAA TCCTCAAACC AACAAAACTC AGTATACACA GCCGCTATTC   3313
ATTGAGGGCT GGATACCTCA ACAAGACTGA GAGCCTTTCC CCGCTTCTCT   3363
CCAAGAAGGA GACGTTCAGC TAGATTTGTT CCCATTTCCG TTGTGTTAAT   3413
TCAAAGCTCA TGCTCCCCTA CGGTACAGGC TGAGGTACAC GGTTAGCAAA   3463
ACCATGGGAA GGGGAATGGC GGTGCATATC ATTAACTAAC ACTCCAAACA   3513
AAGGTGAGCT TGCCCAGGAC TTGGCATTTC CAAATCAAAG TTTTTAGATA   3563
TGAACACCTA CTGTGAGTTC TGCTACAAAG CACAAATGAA TTTGTCTCAA   3613
CTATGCAATT TGATTGGAAA AATGTATGTG CAGCATGTTA CATTTACTTT   3663
CACGGAATAA AGCAGATATG TTTCTGAAA                          3692
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OATP-RP5 (SEQ ID NOS:9 and 10):

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CGCAAAGAAA TGGCTCAAAA GCTTCAGCTC TTTCTGTGCC CTGGGAGCTG     50
AGATGCACGT CAGTGGCCTT GCCAGCGTGG CCAATTCTCT GCTGACTGCC    100
AGAAAAAAGA GGCCAGGAAG AAAGAGGAAA GAGAAGAGAT CGCTCAGGGG    150
TGAGACCATG CCCTTCATCT TTTCTTTTCC CTAATCTCCT CTGCTTGTGT    200
CCACCCACAC TCTCCCCACC TGGCAAAATT GTTCAAAATT GCTGTGGAGT    250
TTACCTCAGT TTCCTCTTTC AGTCTGTGGT GTGTGGTCCA TCCTCTTGCT    300
GAGCACATTG AAAGGAACTG GCTATCTTTG ATCTCTTCCT CCAGATCAGA    350
GTCAAGGAAT GTGTTTATA ATG GAC ACT TCA TCC AAA GAA AAT ATC  396
                      M   D   T   S   S   K   E   N   I

CAG TTG TTC TGC AAA ACT TCA GTG CAA CCT GTT GGA AGG CCT    438
 Q   L   F   C   K   T   S   V   Q   P   V   G   R   P

TCT TTT AAA ACA GAA TAT CCC TCC TCA GAA GAA AAG CAA CCA    480
 S   F   K   T   E   Y   P   S   S   E   E   K   Q   P

TGC TGT GGT GAA CTA AAG GTG TTC TTG TGT GCC TTG TCT TTT    522
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C   C   G   E   L   K   V   F   L   C   A   L   S   F

GTT TAC TTT GCC AAA GCA TTG GCA GAA GGC TAT CTG AAG AGC    564
V   Y   F   A   K   A   L   A   E   G   Y   L   K   S

ACC ATC ACT CAG ATA GAG AGA AGG TTT GAT ATC CCT TCT TCA    606
T   I   T   Q   I   E   R   R   F   D   I   P   S   S

CTG GTG GGA GTT ATT GAT GGT AGT TTT GAA ATT GGG AAT CTC    648
L   V   G   V   I   D   G   S   F   E   I   G   N   L

TTA GTT ATA ACA TTT GTT AGC TAC TTT GGA GCC AAA CTT CAC    690
L   V   I   T   F   V   S   Y   F   G   A   K   L   H

AGG CCA AAA ATA ATT GGA GCA GGG TGT GTA ATC ATG GGA GTT    732
R   P   K   I   I   G   A   G   C   V   I   M   G   V

GGA ACA CTG CTC ATT GCA ATG CCT CAG TTC TTC ATG GAG CAG    774
G   T   L   L   I   A   M   P   Q   F   F   M   E   Q

TAC AAA TAT GAG AGA TAT TCT CCT TCC TCC AAT TCC ACT CTC    816
Y   K   Y   E   R   Y   S   P   S   S   N   S   T   L

AGC ATC TCT CCG TGT CTC CTA GAG TCA AGC AGT CAA TTA CCA    858
S   I   S   P   C   L   L   E   S   S   S   Q   L   P

GTT TCA GTT ATG GAA AAA TCA AAA TCC AAA ATA AGT AAC GAA    900
V   S   V   M   E   K   S   K   S   K   I   S   N   E

TGT GAA GTG GAC ACT AGC TCT TCC ATG TGG ATT TAT GTT TTC    942
C   E   V   D   T   S   S   S   M   W   I   Y   V   F

CTG GGC AAT CTT CTT CGT GGA ATA GGA GAA ACT CCC ATT CAG    984
L   G   N   L   L   R   G   I   G   E   T   P   I   Q

CCT TTG GGC ATT GCC TAC CTG GAT GAT TTT GCC AGT GAA GAC   1026
P   L   G   I   A   Y   L   D   D   F   A   S   E   D

AAT GCA GCT TTC TAT ATT GGG TGT GTG CAG ACG GTT GCA ATT   1068
N   A   A   F   Y   I   G   C   V   Q   T   V   A   I

ATA GGA CCA ATC TTT GGT TTC CTG TTA GGC TCA TTA TGT GCC   1110
I   G   P   I   F   G   F   L   L   G   S   L   C   A

AAA CTA TAT GTT GAC ATT GGC TTT GTA AAC CTA GAT CAC ATA   1152
K   L   Y   V   D   I   G   F   V   N   L   D   H   I

ACC ATT ACC CCA AAA GAT CCC CAG TGG GTA GGA GCC TGG TGG   1194
T   I   T   P   K   D   P   Q   W   V   G   A   W   W

CTT GGC TAT CTA ATA GCA GGA ATC ATA AGT CTT CTT GCA GCT   1236
L   G   Y   L   I   A   G   I   I   S   L   L   A   A

GTG CCT TTC TGG TAT TTA CCA AAG AGT TTA CCA AGA TCC CAA   1278
V   P   F   W   Y   L   P   K   S   L   P   R   S   Q
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AGT AGA GAG GAT TCT AAT TCT TCC TCT GAG AAA TCC AAG TTT 1320
S R E D S N S S S E K S K F

ATT ATA GAT GAT CAC ACA GAC TAC CAA ACA CCC CAG GGA GAA 1362
I I D D H T D Y Q T P Q G E

AAT GCA AAA ATA ATG GAA ATG GCA AGA GAT TTT CTT CCA TCA 1404
N A K I M E M A R D F L P S

CTG AAG AAT CTT TTT GGA AAC CCA GTA TAC TTC CTA TAT TTA 1446
L K N L F G N P V Y F L Y L

TGT ACA AGC ACT GTT CAG TTC AAT TCT CTG TTC GGC ATG GTG 1488
C T S T V Q F N S L F G M V

ACG TAC AAA CCA AAG TAC ATT GAG CAG CAG TAT GGA CAG TCA 1530
T Y K P K Y I E Q Q Y G Q S

TCC TCC AGG GCC AAC TTT GTG ATC GGG CTC ATC AAC ATT CCA 1572
S S R A N F V I G L I N I P

GCA GTG GCC CTT GGA ATA TTC TCT GGG GGG ATA GTT ATG AAA 1614
A V A L G I F S G G I V M K

AAA TTC AGA ATC AGT GTG TGT GGA GCT GCA AAA CTC TAC TTG 1656
K F R I S V C G A A K L Y L

GGA TCA TCT GTC TTT GGT TAC CTC CTA TTT CTT TCC CTG TTT 1698
G S S V F G Y L L F L S L F

GCA CTG GGC TGT GAA AAT TCT GAT GTG GCA GGA CTA ACT GTC 1740
A L G C E N S D V A G L T V

TCC TAC CAA GGA ACC AAA CCT GTC TCT TAT CAT GAA CGA GCT 1782
S Y Q G T K P V S Y H E R A

CTC TTT TCA GAT TGC AAC TCA AGA TGC AAA TGT TCA GAG ACA 1824
L F S D C N S R C K C S E T

AAA TGG GAA CCC ATG TGC GGT GAA AAT GGA ATC ACA TAT GTA 1866
K W E P M C G E N G I T Y V

TCA GCT TGT CTT GCT GGT TGT CAA ACC TCC AAC AGG AGT GGA 1908
S A C L A G C Q T S N R S G

AAA AAT ATT ATA TTT TAC AAC TGC ACT TGT GTG GGA ATT GCA 1950
K N I I F Y N C T C V G I A

GCT TCT AAA TCC GGA AAT TCC TCA GGC ATA GTG GGA AGA TGT 1992
A S K S G N S S G I V G R C

CAG AAA GAC AAT GGA TGT CCC CAA ATG TTT CTG TAT TTC CTT 2034
Q K D N G C P Q M F L Y F L

GTA ATT TCA GTC ATC ACA TCC TAT ACT TTA TCC CTA GGT GGC 2076

V I S Y I T S Y T L S L G G

ATA CCT GGA TAC ATA TTA CTT CTG AGG TGC ATT AAG CCA CAG 2118
I P G Y I L L L R C I K P Q

CTT AAG TCT TTT GCC TTG GGT ATC TAC ACA TTA GCA ATA AGA 2160
L K S F A L G I Y T L A I R

GTT CTT GCA GGA ATC CCA GCT CCA GTG TAT TTT GGA GTT TTG 2202
V L A G I P A P V Y F G V L

ATT GAT ACT TCA TGC CTC AAA TGG GGA TTT AAA AGA TGT GGA 2244
I D T S C L K W G F K R C G

AGT AGA GGA TCA TGC AGA TTA TAT GAT TCA AAT GTC TTC AGA 2286
S R G S C R L Y D S N V F R

CAT ATA TAT TTG GGA CTA ACT GTG ATA CTG GGC ACA GTG TCA 2328
H I Y L G L T V I L G T V S

ATT CTC CTA AGC ATT GCA GTA CTT TTC ATT TTA AAG AAA AAT 2370
I L L S I A V L F I L K K N

TAT GTT TCA AAA CAC AGA AGT TTT ATA ACC AAG AGA GAA AGA 2412
Y V S K H R S F I T K R E R

ACA ATG GTG TCT ACA AGA TTC CAA AAG GAA AAT TAC ACT ACA 2454
T M V S T R F Q K E N Y T T

AGT GAT CAT CTG CTA CAA CCC AAC TAC TGG CCA GGC AAG GAA 2496
S D H L L Q P N Y W P G K E

ACT CAA CTT TAG AAACATGATG ACTGGAAGTC ATGTCTTCTA 2538
T Q L *

ATTGGTTGAC ATTTTGCAAA CAAATAAATT GTAATCAAAA GAGCTCTAAA 2588
TTTGTAATTT CTTTCTCCTT TCAAAAAATG TCTACTTTGT TTTGGTCCTA 2638
GGCATTAGGT AATATAACTG ATAATATACT GAAATATATA ATGGAAGATG 2688
CAGATGATAA AACTAATTTT GAACTTTTTA ATTTATATAA ATTATTTTAT 2738
ATCATTTACT TATTTCACTT TATTTTGCTT TGTGCTCATT GATATATATT 2788
AGCTGTACTC CTAGAAGAAC AATTGTCTCT ATTGTCACAC ATGGTTATAT 2838
TTAAAGTAAT TTCTGAACTG TGTAATGTGT CTAGAGTAAG CAAATACTGC 2888
TAACAATTAA CTCATACCTT GGGTTCCTTC AAGTATTACT CCTATAGTAT 2938
TTTCTCCCAT AGCTGTCTTC ATCTGTGTAT TTTAATAATG ATCTTAGGAT 2988
GGAGCAGAAC ATGGAGAGGA AGATTTCATT TTAAGCTCCT CCTTTTCCTT 3038
GAAATACAAT AATTTATATA GAAATGTGTA GCAGCAAATT ATATTGGGGA 3088
TTAGAATTTT GAATTAATAG CTCTCCTACT ATTAATTTAC ATGTGCTTTT 3138
TGTGTGGCGC TATAAGTGAC TATGGTTGTA AAGTAATAAA ATTGATGTTA 3188
ACATGCCCAA TTATTGTTCT TTTATGAATT CAATGAATTT AAAACTATTG 3238
TTAAATATAA TACTGCCCCA CTTTAATATA TGTAAGCAAC TTCCTACTTA 3288
TACACGACGT GTTCCTAAAA CATGTTTGAA AGGTGAATTT CTGAAAGTCT 3338
CCCATAAATG TAGGTGTTAC AACAGGAAAA AAAAAAAAAA AAA 3381

OATP-RP1 (SEQ ID NOS:11 and 12):

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                                           GGCACGAG GCGCTGCGCG    18
GCGCGGCGGC CGGGCCCTCG AGACGGGGAC GGACACACCA GCCCCTCGGA    68
TACCACTTGG CCACTCCCGC TGAGGCCACT CCCACTGCGT GGCTGAAGCC   118
TCGAGGTCAC CAGGCGGAGG CGCGGAG ATG CCC CTG CAT CAG CTG GGG   166
                               M   P   L   H   Q   L   G

GAC AAG CCG CTC ACC TTC CCC AGC CCC AAC TCA GCC ATG GAA   208
 D   K   P   L   T   F   P   S   P   N   S   A   M   E

AAC GGG CTT GAC CAC ACC CCA CCC AGC AGG AGG GCA TCC CCG   250
 N   G   L   D   H   T   P   P   S   R   R   A   S   P

GGC ACA CCC CTG AGC CCC GGG TCC CTC CGC TCC GCT GCC CAT   292
 G   T   P   L   S   P   G   S   L   R   S   A   A   H

AGC CCC CTG GAC ACC AGC AAG CAG CCC CTC TGC CAG CTC TGG   334
 S   P   L   D   T   S   K   Q   P   L   C   Q   L   W

GCC GAG AAG CAT GGC GCC CGG GGG ACC CAT GAG GTG CGG TAC   376
 A   E   K   H   G   A   R   G   T   H   E   V   R   Y

GTC TCG GCC GGG CAG AGC GTG GCG TGC GGC TGG TGG GCC TTC   418
 V   S   A   G   Q   S   V   A   C   G   W   W   A   F

GCA CCG CCG TGC CTG CAG GTC CTC AAC ACG CCC AAG GGC ATC   460
 A   P   P   C   L   Q   V   L   N   T   P   K   G   I

CTG TTC TTC CTG TGT GCG GCC GCA TTC CTG CAG GGG ATG ACT   502
 L   F   F   L   C   A   A   A   F   L   Q   G   M   T

GTG AAT GGC TTC ATC AAC ACA GTC ATC ACC TCC CTG GAG CGC   544
 V   N   G   F   I   N   T   V   I   T   S   L   E   R

CGC TAT GAC CTG CAC AGC TAC CAG AGC GGG CTC ATC GCC AGC   586
 R   Y   D   L   H   S   Y   Q   S   G   L   I   A   S

TCC TAC GAC ATT GCC GCC TGC CTC TGC CTC ACC TTC GTC AGC   628
 S   Y   D   I   A   A   C   L   C   L   T   F   V   S

TAC TTC GGG GGC TCA GGG CAC AAG CCG CGC TGG CTG GGC TGG   670
 Y   F   G   G   S   G   H   K   P   R   W   L   G   W

GGC GTG CTG CTT ATG GGC ACG GGG TCG CTG GTG TTC GCG CTG   712
 G   V   L   L   M   G   T   G   S   L   V   F   A   L

CCC CAC TTC ACG GCT GGC CGC TAT GAG GTG GAG TTG GAC GCG   754
 P   H   F   T   A   G   R   Y   E   V   E   L   D   A

GGT GTC AGG ACG TGC CCT GCC AAC CCC GGC GCG GTG TGT GCG   796
 G   V   R   T   C   P   A   N   P   G   A   V   C   A

GAC AGC ACC TCG GGC CTG TCC CGC TAC CAG CTG GTC TTC ATG   838
 D   S   T   S   G   L   S   R   Y   Q   L   V   F   M
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CTG GGC CAG TTC CTG CAT GGC GTG GGT GCC ACA CCC CTC TAC 880
L G Q F L H G V G A T P L Y

ACG CTG GGC GTC ACC TAC CTG GAT GAG AAC GTC AAG TCC AGC 922
T L G V T Y L D E N V K S S

TGC TCG CCC GTC TAC ATT GCC ATC TTC TAC ACA GCG GCC ATC 964
C S P V Y I A I F Y T A A I

CTG GGC CCA GCT GCC GGC TAC CTG ATT GGA GGT GCC CTG CTG 1006
L G P A A G Y L I G G A L L

AAT ATC TAC ACG GAA ATG GGC CGA CGG ACG GAG CTG ACC ACC 1048
N I Y T E M G R R T E L T T

GAG AGC CCA CTG TGG GTC GGC GCC TGG TGG GTC GGC TTC CTG 1090
E S P L W V G A W W V G F L

GGC TCT GGG GCC GCT GCT TTC TTC ACC GCC GTT CCC ATC CTT 1132
G S G A A A F F T A V P I L

GGT TAC CCT CGG CAG CTG CCA GGC TCC CAG CGC TAC GCG GTC 1174
G Y P R Q L P G S Q R Y A V

ATG AGA GCG GCG GAA ATG CAC CAG TTG AAG GAC AGC AGC CGT 1216
M R A A E M H Q L K D S S R

GGG GAG GCG AGC AAC CCG GAC TTT GGG AAA ACC ATC AGA GAC 1258
G E A S N P D F G K T I R D

CTG CCT CTC TCC ATC TGG CTC CTG CTG AAG AAC CCC ACG TTC 1300
L P L S I W L L L K N P T F

ATC CTG CTC TGC CTG GCC GGG GCC ACC GAG GCC ACT CTC ATC 1342
I L L C L A G A T E A T L I

ACC GGC ATG TCC ACG TTC AGC CCC AAG TTC TTG GAG TCC CAG 1384
T G M S T F S P K F L E S Q

TTC AGC CTG AGT GCC TCA GAA GCT GCC ACC TTG TTT GGG TAC 1426
F S L S A S E A A T L F G Y

CTG GTG GTG CCA GCG GGT GGT GGC GGC ACC TTC CTG GGC GGC 1468
L V V P A G G G G T F L G G

TTC TTT GTG AAC AAG CTC AGG CTC CGG GGC TCC GCG GTC ATC 1510
F F V N K L R L R G S A V I

AAG TTC TGC CTG TTC TGC ACC GTT GTC AGC CTG CTG GGC ATC 1552
K F C L F C T V V S L L G I

CTC GTC TTC TCA CTG CAC TGC CCC AGT GTG CCC ATG GCG GGC 1594
L V F S L H C P S V P M A G

GTC ACA GCC AGC TAC GGC GGG AGC CTC CTG CCC GAA GGC CAC 1636

V T A S Y G G S L L P E G H

CTG AAC CTA ACG GCT CCC TGC AAC GCT GCC TGC AGC TGC CAG 1678

L N L T A P C N A A C S C Q

CCA GAA CAC TAC AGC CCT GTG TGC GGC TCG GAC GGC CTC ATG 1720

P E H Y S P V C G S D G L M

TAC TTC TCA CTG TGC CAC GCA GGG TGC CCT GCA GCC ACG GAG 1762

Y F S L C H A G C P A A T E

ACG AAT GTG GAC GGC CAG AAG GTG TAC CGA GAC TGT AGC TGT 1804

T N V D G Q K V Y R D C S C

ATC CCT CAG AAT CTT TCC TCT GGT TTT GGC CAT GCC ACT GCA 1846

I P Q N L S S G F G H A T A

GGG AAA TGC ACT TCA ACT TGT CAG AGA AAG CCC CTC CTT CTG 1888

G K C T S T C Q R K P L L L

GTT TTC ATA TTC GTT GTA ATT TTC TTT ACA TTC CTC AGC AGC 1930

V F I F V V I F F T F L S S

ATT CCT GCA CTA ACG GCA ACT CTA CGA TGT GTC CGT GAC CCT 1972

I P A L T A T L R C V R D P

CAG AGA TCC TTT GCC CTG GGA ATC CAG TGG ATT GTA GTT AGA 2014

Q R S F A L G I Q W I V V R

ATA CTA GGG GGC ATC CCG GGG CCC ATC GCC TTC GGC TGG GTG 2056

I L G G I P G P I A F G W V

ATC GAC AAG GCC TGT CTG CTG TGG CAG GAC CAG TGT GGC CAG 2098

I D K A C L L W Q D Q C G Q

CAG GGC TCC TGC TTG GTG TAC CAG AAT TCG GCC ATG AGC CGC 2140

Q G S C L V Y Q N S A M S R

TAC ATA CTC ATC ATG GGG CTC CTG TAC AAG GTG CTG GGC GTC 2182

Y I L I M G L L Y K V L G V

CTC TTC TTT GCC ATA GCC TGC TTC TTA TAC AAG CCC CTG TCG 2224

L F F A I A C F L Y K P L S

GAG TCT TCA GAT GGC CTG GAA ACT TGT CTG CCC AGC CAG TCC 2266

E S S D G L E T C L P S Q S

TCA GCC CCT GAC AGT GCC ACA GAT AGC CAG CTC CAG AGC AGC 2308

S A P D S A T D S Q L Q S S

GTC TGA CCACCGCCCG CGCCCACCCG GCCACGGCGG GCACTCAGCA 2354

V *

TTTCCTGATG ACAGAACAGT GCCGTTGGGT GATGCAATCA CACGGGAACT 2404

TCTATTTGAC CTGCAACCTT CTACTTAACC TGTGGTTTAA AGTCGGCTGT 2454

GACCTCCTGT CCCCAGAGCT GTACGGCCCT GCAGTGGGTG GGAGGAACTT 2504

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GCATAAATAT ATATTTATGG ACACACAGTT TGCATCAGAA CGTGTTTATA    2554
GAATGTGTTT TATACCCGAT CGTGTGTGGT GTGCGTGAGG ACAAACTCCG    2604
CAGGGGCTGT GAATCCCACT GGGAGGGCGG CGGGCCTGCA GCCCGAGGAA    2654
GGCTTGTGTG TCCTCAGTTA AAACTGTGCA TATCGAAATA TATTTTGTTA    2704
TTTAAGCCTG CGAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA    2754
AAAAAAAAA                                                2763
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Persons skilled in the art can also modify the nucleic acids coding for the OATPs of the present invention to prepare useful mutations. For example, one may modify the sequence to provide additional restriction endonuclease recognition sites in the nucleic acid. Such mutations may be silent or may change the amino acid encoded by the mutated codon. One can prepare these modified nucleic acids, for example, by mutating the nucleic acid coding for an OATP of the present invention to result in deletion, substitution, insertion, inversion or addition of one or more amino acids in the encoded polypeptide. For methods of site-directed mutagenesis, see Taylor, J. W. et al. (1985), Nucl. Acids Res. 13, 8749-64 and Kunkel, J. A. (1985), Proc. Natl. Acad. Sci. USA 82: 482-92. In addition, kits for site-directed mutagenesis are available from commercial vendors (e.g., BioRad Laboratories, Richmond, CA; Amersham Corp., Arlington Heights, IL). For disruption, deletion and truncation methods, see Sayers, J. R. et al. (1988), Nucl. Acids Res. 16: 791-800.

This invention also comprises modified nucleic acids, including (1) alternative splice exon variants; (2) allelic variants; and (3) chimeric proteins in which the fusion construct comprises an OATP or fragment thereof. Such modified nucleic acids can be obtained by persons of ordinary skill in the art when armed with the present disclosure.

Expression vectors

This invention further concerns expression vectors comprising a nucleotide sequence encoding an OATP of the present invention. Preferably, the expression vectors comprise all or a portion of the nucleic acid sequence as shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11; preferred is a nucleotide sequence encoding an OATP as shown above (i.e., the coding region).

Expression vectors are usually plasmids, but the invention includes other vector forms that serve equivalent functions and become known in the art

subsequently hereto. A person skilled in the art might also stably integrate a sequence encoding an OATP into the chromosome of an appropriate host cell.

Expression vectors typically contain regulatory elements capable of affecting expression of an OATP. These regulatory elements can be heterologous or native OATP elements. Typically, a vector contains an origin of replication, a promoter, and a transcription termination sequence. The vector may also include other regulatory sequences, including mRNA stability sequences, which provide for stability of the expression product; secretory leader sequences, which provide for secretion of the expression product; environmental feedback sequences, which allow expression of the structural gene to be modulated (e.g., by the presence or absence of nutrients or other inducers in the growth medium); marking sequences, which are capable of providing phenotypic selection in transformed host cells; restriction sites, which provide sites for cleavage by restriction endonucleases; and sequences which allow expression in various types of hosts, including prokaryotes, yeasts, fungi, plants and higher eukaryotes.

An expression vector of this invention is at least capable of directing the replication, and preferably the expression, of the nucleic acids and protein of this invention. Suitable origins of replication include, for example, the Col E1, the SV4O viral, Epstein Barr viral, and the M13 origins of replication. Suitable promoters include, for example, the cytomegalovirus promoter, the lacZ promoter, the gal10 promoter and the Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) polyhedral promoter. Suitable termination sequences include, for example, the bovine growth hormone, SV40, lacZ and AcMNPV polyhedral polyadenylation signals. Examples of selectable markers include neomycin, ampicillin, and hygromycin resistance and the like.

Persons skilled in the art may insert DNA encoding An OATP of the present invention into several commercially available vectors. Examples include vectors compatible with mammalian cells, such as pcDNA3 or pCEP4; baculovirus vectors such as pBlueBac; prokaryotic vectors such as pcDNA2; and yeast vectors such as pYes2. For vector modification techniques, see Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Host cells

This invention additionally concerns host cells containing an expression vector that comprises a sequence encoding an OATP, preferably the OATP2, OATP-RP2, OATP-RP3, OATP-RP4, OATP-RP5 or OATP-RP1 of the present invention. The host cells preferably contain an expression vector which comprises all or part of the DNA sequence having the nucleotide sequence substantially as shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11, particularly the coding regions thereof. Suitable host cells include both prokaryotic cells (e.g., E. coli strains HB101, DH5a, XL1 Blue, Y1090 and JM101) and eukaryotic cells (e.g., Spodoptera frugiperda insect cells, CHO cells, COS-7 cells, HEK 293 cells, human skin fibroblasts, and S. cerevisiae cells).

Persons skilled in the art may introduce expression vectors into host cells by various methods known in the art. Exemplary methods are transfection by calcium phosphate precipitation, electroporation, liposomal fusion, nuclear injection, and viral or phage infection. One may then culture the host cell under conditions permitting expression of large amounts of OATP.

One may identify such modified host cells by any of five general approaches:

(a) DNA-DNA hybridization with probes complementary to the sequence encoding an OATP (Southern blotting).

(b) detection of marker gene functions, such as thymidine kinase activity, resistance to antibiotics, and the like. A marker gene can be placed in the same plasmid as an OATP sequence under the regulation of the same or a different promoter.

(c) detection of mRNA transcripts by hybridization assays (e.g., Northern blotting or a nuclease protection assay using a probe complementary to the RNA sequence).

(d) immunodetection of gene expression (e.g., by Western blotting with antibody to OATP).

(e) PCR with primers homologous to expression vector sequences or sequences encoding OATP. The PCR produces a DNA fragment of predicted length, indicating incorporation of the expression system in the host cell.

Persons skilled in the art may determine DNA sequences by various known methods. See, for example, the dideoxy chain termination method in Sanger et al. (1977), Proc. Natl. Acad. Sci. USA 74: 5463-7 and the Maxam-Gilbert method in Maxam-Gilbert (1977), Proc. Natl. Acad. Sci. USA 74: 560-4.

One may use the host cells of this invention in a variety of ways that are now apparent. One may use the cells to screen for compounds that bind to or otherwise modulate or regulate the function of an OATP of the present invention, which would be useful for modulation, for example activation or inactivation, of OATP2, OATP-RP2, OATP-RP3, OATP-RP4, OATP-RP5 or OATP-RP1 activity; to study signal transduction mechanisms and protein-protein interactions; and to prepare OATP for the uses described below.

Not all expression vectors and DNA regulatory sequences will function equally well to express the DNA sequences of this invention. Neither will all host cells function equally well with the same expression system. However, one of ordinary skill in the art may make a selection among expression vectors, DNA regulatory sequences, and host cells using the guidance provided herein without undue experimentation and without departing from the scope of the invention.

Polypeptides

This invention further concerns polypeptides comprising all or a portion of the amino acid sequences of OATPs of the present invention. The inventors prefer polypeptides comprising all or a portion of the amino acid sequences shown as in SEQ ID NO:2 (OATP2), SEQ ID NO:4 (OATP-RP2), SEQ ID NO:6 (OATP-RP3), SEQ ID NO:8 (OATP-RP4), SEQ ID NO:10 (OATP-RP5) or SEQ ID NO:12 (OATP-RP1). Where a portion of an OATP of the present invention is used, preferably the portion exhibits the same biological activity of the OATP from which the portion is derived. For example, and within the scope of the invention, are polypeptides that comprise all or a portion of OATP2, OATP-RP2, OATP-RP3, OATP-RP4, OATP-RP5 or OATP-RP1 that exhibit transport activity. The portions may contain one or more mutations so that the protein(s) fail(s) to exhibit transport activity, but that can be used to screen for compounds that will modulate or bind to the protein or portion thereof.

Persons having ordinary skill in the art may prepare these polypeptides by methods known in the art. For example, one may use chemical synthesis, such as the solid phase procedure described by Houghton et al. (1985), Proc. Natl. Acad. Sci. 82: 5131-5. Another method is in vitro translation of mRNA. One may also produce the polypeptides in the above-described host cells, which is the preferred method. For example, one may synthesize DNA comprising all or a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11 by PCR as described above, insert the synthesized DNA into an expression vector, transform a host cell with the expression vector, and culture the host cell to produce the desired polypeptides.

Persons skilled in the art can isolate and purify such polypeptides by any one of several known techniques: for example, ion exchange chromatography, gel filtration chromatography and affinity chromatography. Such techniques may require modification of the protein. For example, one may add a histidine tag to the protein to enable purification on a nickel column.

Persons skilled in the art can use the polypeptides of the invention in a wide variety of ways. For example, one may use them to generate polyclonal or monoclonal antibodies. One may then use such antibodies for immunodetection (e.g., radioimmunoassay, enzyme immunoassay, or immunocytochemistry), immunopurification (e.g., affinity chromatography) of polypeptides from various sources, or immunotherapy.

Persons skilled in the art may make modified OATP polypeptides by known techniques. Such modifications may cause higher or lower activity, permit higher levels of protein production, or simplify purification of the protein. Such modifications may help identify specific OATP amino acids involved in binding, which in turn may help rational drug design of OATP modulators. One can make amino acid substitutions based on similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups or nonpolar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine, glycine, alanine; asparagine,

glutamine; serine, threonine; phenylalanine, tyrosine. All such modified polypeptides are included within the scope of the invention.

Preferred analogs include proteins that differ from the novel OATPs of the present invention (or biologically active fragments thereof) by one or more conservative amino acid substitutions or by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the biological activity of the analog. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Other conservative amino acid substitutions can be taken from the table below.

Table 1
Conservative amino acid replacements

For Amino Acid	Code	Replace with any of:
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, ß-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-1-thioazolidine-4-carboxylic acid, D- or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Other analogs within the invention are those with modifications which increase protein or peptide stability; such analogs may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the protein or peptide sequence. Also included are analogs that include residues other than naturally occurring L-amino acids. e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., ß or γ amino acids.

The inventors contemplate a number of other variations of the above-described polypeptides. Such variations include salts and esters of the polypeptides. as well as precursors of the aforementioned polypeptides (e.g., having N-terminal substituents such as methionine, N-formylmethionine and leader sequences). The invention includes all such variations.

Method for detecting nucleic acids

The present invention further concerns a method for detecting nucleic acids encoding OATP proteins. In this method, a person of ordinary skill in the art (a) contacts nucleic acids of unknown sequence with a nucleic acid having a sequence complementary to a known coding sequence (e.g., a sequence of at least about 10 nucleotides from, e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11, particularly the coding regions thereof), wherein the latter nucleic acid has a detectable marker; and (b) determines the presence of marker bound to any of the nucleic acids of unknown sequence. The presence of bound marker indicates the presence of the desired nucleic acids. One can apply this method to detect OATP nucleic acids from other tissues (which may have different regulatory elements) and nucleic acids from other species (e.g., monkey).

Persons of ordinary skill in the art generally know how to obtain nucleic acids to be analyzed in this method. For genomic DNA, one can rapidly freeze tissue, crush the tissue into readily digestible pieces, and incubate the crushed tissue in proteinase K and SDS to degrade most cellular proteins. One can then deproteinize the genomic DNA by successive phenol/chloroform/isoamyl alcohol extractions. recover DNA by ethanol precipitation, dry it and resuspend it in buffer. For RNA. one can lyse cultured cells in 4M guanidinium solution, draw the lysate through a 20-gauge needle, pellet the RNA through a cesium chloride step gradient. and remove the supernatant. The pellet should contain purified RNA.

The detectable marker may be a radioactive ion linked to one of the nucleotides of the complementary nucleic acid. Common radioactive labels are ^{32}P and ^{35}S, although one may also use other labels such as biotin. Persons skilled in the art are aware of various methods to attach the labels to the complementary nucleic acid (e.g., the random primer method for attachment of ^{32}P or ^{35}S).

Persons of ordinary skill in the art generally know how to carry out such a method of detecting nucleic acids. For example, one may perform a Southern or northern blot using a radiolabeled OATP complementary oligonucleotide probe. One can then detect hybridization by autoradiography. Depending on the marker, one may also use other detection methods (e.g., spectrophotometry).

Methods for detecting OATP modulators and compounds transported by the OATPs of the present invention

This invention further concerns methods for detecting modulators of the OATPs of the present invention, as well as methods for detecting compounds that are transported by the OATPs of the present invention (e.g., compounds that are transported into the liver that may be used as carriers for other compounds). A screen for OATP modulators entails detecting binding of molecules (e.g., polypeptides, natural products, synthetic compounds) in cells expressing OATP protein. Alternatively, a screen for OATP positive modulators and/or negative modulators entails detecting the augmentation and/or inhibition of transport of a known compound. A screen for OATP-transported compounds entails detecting the transport of molecules (e.g., polypeptides, natural products, synthetic compounds) by an OATP.

Cloning and sequencing of the OATPs of the present invention enables construction of cells useful in screening for natural products and synthetic compounds that bind to, modulate, and/or are transported by OATP activity. A process for detecting OATP modulators requires transforming a suitable vector into compatible host cells as described previously herein. One treats such transformed cells with test substances (e.g., synthetic compounds or natural products), and then measures activity in the presence and absence of the test substance.

OATP Assay

An assay for the measurement of OATP activity is performed as follows: HEK293 cells are plated in Dulbeccos Modified Eagles Medium (DMEM) plus 10%

fetal bovine serum plus penecillin and streptomycin. in poly-d-lysine coated dishes and co-transfected with OATP transporter expression plasmids using Lipofectamine Plus (Life Technologies. Inc.). The cells and media are assayed for substrate transport 24 hours later. Alternatively, cell lines engineered to stably express OATPs could be plated and assayed directly without transfection. To measure transport. media is removed and monolayers are assayed in triplicate by washing once in serum-free DMEM and adding the same medium containing [^{3}H]-substrate alone or in the presence of various concentrations of unlabeled test compounds. For OATP2, the [^{3}H]-substrate could be [^{3}H]-pravastatin, [^{3}H]-taurocholate, or [^{3}H]-dehydroepiandrosterone sulfate, or [^{125}I]-thyroid hormone (T4). Monolayers are incubated at room temperature for 5 to 10 minutes depending on the transporter. Then the cells are rapidly washed once with ice cold DMEM containing 5% BSA, twice with DMEM plus 0.1% BSA and once with DMEM alone. Cells are lysed in 0.1 N NaOH and a fraction of the lysate is used to determine radiolabel incorporation by liquid scintillation counting, and another is used to determine protein concentration in the lysate using the Bradford assay with BSA as a standard. The transport activity is expressed as moles of substrate transported into cells/mg of cell protein/minute.

Drug Targeting

Also included within the present invention is tissue expression of an OATP of the present invention. The OATPs of the present invention are also useful for targeting drugs to certain organs that express an OATP described herein (e.g., the liver), and for modulating the concentration of endogenous substrates.

For example, the novel organic anion transporter disclosed herein. OATP2, represents a potential therapeutic target due to its ability to modulate the cellular uptake and potential secretion of a several biologically important organic anions, including bile acids and the androgen hormone dehydroepiandrosterone sulfate ("DHEAS"). Furthermore. since OATP2 transports at least one drug (i.e. pravastatin), and other members of this family are known to transport a variety of other xenobiotics. this transporter could be exploited to optimize the delivery of drugs into liver and away from other tissues.

OATP2 is unique among the OATP family, in that it is the only known organic anion transporter that is expressed exclusively in the liver. Thus. drugs

optimized for this transporter could be targeted for hepatic delivery with greater selectivity than with any other known transporter. To generalize this approach, it may be possible to identify a small molecule "adaptor" that is efficiently recognized and transported by OATP2 (an OATP2-transported compound) that could be appended to other drugs for hepatic targeting even if the parent compound is not transported by OATP2.

Alternatively, if a therapeutic compound is taken up into the liver entirely or substantially by OATP2, one could inhibit hepatic clearance and thereby elevate circulating concentrations, or increase the compounds half-life in the periphery, by adding a functionality to said compound that disallows transport by OATP2. Likewise, if an endogenous substance utilizes OATP2 for liver uptake and clearance from the circulation, a competitive or non-competitive OATP2 inhibitor could elevate plasma levels of said substance. As an example, DHEAS is an adrenal androgen that declines with age and on the basis of some animal data, it has been suggested that replacement of DHEAS deficiency may stimulate age-related immune deficiencies, increase cognitive function and insulin sensitivity, and maintain bone mass. Inhibiting the hepatic clearance of endogenous DHEAS through blocking its interactions with OATP2 could result in elevated hormone levels in the absence of hormone supplementation.

With the information provided herein, one skilled in the art is able to identify molecules, both naturally occurring and synthetic (including therapeutic drugs), that are transported by the OATPs, e.g., OATP2, disclosed herein. OATPs as a class generally exhibit broad substrate specificity ("polyspecific" transporters). Thus, it is anticipated that many additional substrates of these transporters will be identified.

Gene Therapy

Persons skilled in the art can also use sense and antisense nucleic acid molecules as therapeutic agents for OATP-related indications. One may construct vectors that direct the synthesis of the desired DNA or RNA or formulate the nucleic acid as described in the art.

Several references describe the usefulness of antisense molecule. See Toulme and Helene (1988), Gene 72: 51-8; Inouye (1988), Gene, 72: 25-34; Uhlmann and Peyman (1990), Chemical Reviews 90: 543-584; Biotechnology Newswatch (January

15, 1996), p. 4; Robertson, Nature Biotechnology 15: 209 (1997); Gibbons and Dzau (1996), Science 272: 689-93. One can design them based on genomic DNA and/or cDNA, 5' and 3' flanking control regions, other flanking sequences, intron sequences, and nonclassic Watson and Crick base pairing sequences used in formation of triplex DNA. Such antisense molecules include antisense oligodeoxyribonucleotides, oligoribonucleotides, oligonucleotide analogues, and the like, and may comprise at least about 15 to 25 bases.

Antisense molecules may bind noncovalently or covalently to the OATP DNA or RNA. Such binding could, for example, cleave or facilitate cleavage of OATP DNA or RNA, increase degradation of nuclear or cytoplasmic mRNA, or inhibit transcription, translation, binding of transactivating factors, or pre-mRNA splicing or processing. Antisense molecules may also contain additional functionalities that increase stability, transport into and out of cells, binding affinity, cleavage of the target molecule, and the like. All of these effects would decrease expression of OATP protein and thus make the antisense molecules useful as OATP modulators.

EXAMPLES

The following examples are included for understanding the present invention and are not intended to limit the scope of Applicants invention, which is defined solely by the claims.

Example 1

Isolation of OATP2, OATP-RP1, OATP-RP2, OATP-RP3, OATP-RP4 and OATP-RP5 full length cDNAs and cloning into mammalian expression vectors

Human OATP2 was identified by searching the public EST databases for sequences homologous to human OATP. One EST sequence, Genbank accession number T73863, encoded a partial cDNA with significant sequence identity with OATP. EST sequences encoding partial cDNAs for OATP-RP1, OATP-RP2, OATP-RP3, OATP-RP4, and OATP-RP5 were identified by searching the public EST databases and the Incyte, Inc. EST database for sequences homologous to human OATP. The EST clone IDs corresponding to OATP-RP1 are 820117, 2668489, 1610706, 2972518, and 588148. These clones represent a contig encoding only part of the full length cDNA. The Incyte EST clone IDs corresponding to OATP-RP2 are

1664737 and 2641944. These clones represent a contig encoding only part of the full length cDNA. The Incyte EST clone IDs corresponding to OATP-RP3 are 2493241, 2497845, and 2664024. These clones represent a contig encoding only part of the full length cDNA. The Incyte EST clone IDs corresponding to OATP-RP4 are 1494683 and 1685219. These clones represent a contig encoding only part of the full length cDNA. The Incyte EST clone ID corresponding to OATP-RP5 is 925716. This clone encodes only part of the full length cDNA. Full length clones for each of the above genes were obtained using the Gene Trapper cDNA Positive Selection System (LifeTechnologies, Inc.). In this procedure, a single or multiple oligonucleotides complementary to each of the EST contigs or individual EST sequences, were biotinylated at the 3'-end and used to hybridize to a single-stranded human cDNA library constructed in pCMVSport2 (LifeTechnologies, Inc.). The sequence of oligonucleotides used for each gene as well as the tissue source of the libraries screened are shown in Table 2.

Table 2

Oligonucleotides used to screen for OATP Full length cDNAs using Gene-Trapper Selection

Gene	Biotinylated capture oligonucleotide(s) used	Seq ID number of oligonucleotide	Human cDNA library screened
OATP2	5'-ACCCTGTCTAGCAGGTTGCA-3'	13	liver
OATP-RP1	5'-CTGTCGGAGTCTTCAGATG-3'	14	brain
OATP-RP2	5'-TCCATCACAGCCTCCTACGC-3'	15	liver
OATP-RP3	5'-TGCCTCTACTCTGACCCTAG-3'	16	heart
OATP-RP4	5'-GGAGCAGTCATTGACACCAC-3' 5'-TGCTGGGAGTACAACGTGACG-3' 5'-ACAAGGAGGATGGACTGCAG-3'	17 18 19	heart
OATP-RP5	5'-CAGGAATCCCAGCTCCAGTG-3' 5'-GCTACAACCCAACTACTGGC-3' 5'-GGGACTAACTGTGATACTGG-3'	20 21 22	brain

Hybrids between the biotinylated oligonucleotides and single-stranded cDNA were captured on streptavidin-coated paramagnetic beads. After washing, the captured single-stranded cDNA targets was released from the biotinylated oligonucleotides and converted to dsDNA by DNA polymerase using the corresponding unbiotinylated oligonucleotide. Following transformation and plating, several positive clones for each gene were identified by PCR analysis. Full-length cDNA clones were identified

by sequencing. In the case of OATP-RP1, a partial cDNA was obtained by the above technique (pSP-RP1A). Another cDNA clone that was part of the OATP-RP1 contig was identified by searching the public EST databases (Genbank accession number AI027850). An EcoRI-NotI fragment of this clone containing the first 477 nucleotides of OATP-RP1 (SEQ ID NO: 11) (obtained from Research Genetics, Inc.) was ligated to EcoRI-Not I digested pSP-RP1A to generate the full length sequence.

Two polymorphic positions were identified when sequencing multiple OATP-RP4 cDNA clones. Thus, nucleotide number 713 of SEQ ID NO: 7 can be either a C, encoding Leu in SEQ ID NO:8, or a T, encoding a Phe in SEQ ID NO:8. Similarly, nucleotide number 2397 of SEQ ID NO: 7 can be either a G, encoding a Gly in SEQ ID NO:8 , or a T, encoding a Val in SEQ ID NO:8.

For expression studies, OATP2 cDNA was cloned into the expression vector pCEP4βR, a modified form of pCEP4 (Invitrogen, Inc.) in which the CMV promoter-driven expression cassette has been inverted, and used in transient transfections. To accomplish this, OATP2 cDNA in pCMVSport2, correponding to nucleotides 59 through 2361 of SEQ ID NO:1, was excised by digestion with KpnI and NotI. This fragment was cloned into KpnI-NotI digested pCEP4βR. This clone, pCEP-OATP2 was used for transient transfection expression studies.

Example 2

Tissue and cellular distribution of OATP2, OATP-RP1, OATP-RP2, OATP-RP4, and OATP-RP5

The tissue distribution of OATP2, OATP-RP1, OATP-RP2, OATP-RP4, and OATP-RP5 expression was determined by Northern blotting of poly A+ RNA from a variety of human tissues (Figure 1). Transporters of this family previously described in the literature, namely human OATP, rat oatp1, rat oatp2 and rat oatp3, are all expressed in liver, kidney and brain. All of the above transport bile acids as well as a variety of other substrates that are specific for subsets of these transporters. In contrast, the expression of OATP2, which also transports bile acids, is very hepato-specific; a major 3.2 kb and several minor hybridizing bands were observed only in RNA from liver and no other tissue. The specific cell types that express this transporter were examined by *in situ* hybridization of OATP2 riboprobe to human liver samples. Strong hybridization signal was seen localized to hepatocytes

throughout the liver lobule with no significant difference in signal intensity among centrilobular, midzonal or periportal regions. No signal was observed in bile ducts, Kupffer cells, or blood vessels, nor in any cell types from human lung (data not shown).

OATP-RP1 is expressed in nearly all tissues tested with highest abundance in skeletal muscle, lung, placenta, and heart. OATP-RP2 is ubiquitously expressed in all tissues tested. OATP-RP4 has a much more restricted pattern of expression with abundant transcipts in skeletal muscle and heart and much less in prostate and thymus. The expression of OATP-RP5 is likewise tissue specific, with brain and testes being the only sites where transcripts were detected.

Example 3

Expression of OATP2 in transfected cells

293EBNA cells (Invitrogen, Inc.), an HEK293 cell derivative, were transiently transfected with the OATP2 expression vector pCEP-OATP2, or the pCEP4 vector alone (MOCK) and the transport of [^{3}H]-labeled substrates was determined 24 hours later. Figure 2A shows specific uptake of [^{3}H]-pravastatin and [^{3}H]-DHEAS. Figures 2B and 2C show the specific uptake of [^{3}H]-taurocholate and [125I]-thyroid hormone (T4), repsectively. The uptake of radiolabeled substrate for 5 minutes into cells transfected with pCEP-OATP2 or empty vector (MOCK) was determined in the absence (solid bars) and presence (open bars) of excess unlabeled substrate. Thus, OATP2 is a liver specific human transporter of at least some HMG CoA reductase inhibitors, bile acids, adrenal steroids, and thyroid hormone.

We claim:

1. A purified and isolated nucleic acid sequence encoding all or a portion of an organic anion transport protein ("OATP"), said OATP comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2 (OATP2), SEQ ID NO:4 (OATP-RP2), SEQ ID NO:6 (OATP-RP3), SEQ ID NO:8 (OATP-RP4), SEQ ID NO:10 (OATP-RP5), and SEQ ID NO:12 (OATP-RP1).

2. The nucleic acid sequence of claim 1 comprising (a) a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:11; (b) the coding region of (a); (c) the complement of (a) or (b); or (d) nucleic acid sequences that differ from (a), (b) or (c) due to degeneracy of the genetic code.

3. An expression vector comprising a nucleic acid molecule as claimed in claim 1 or 2 and an expression control sequence operatively linked to the nucleic acid molecule.

4. A transformant host cell including an expression vector comprising a nucleic acid molecule as claimed in claim 1 or 2 and an expression control sequence operatively linked to the nucleic acid molecule.

5. An OATP protein comprising the amino acid sequence selected from the group consisting of SEQ ID NO:2 (OATP2), SEQ ID NO:4 (OATP-RP2), SEQ ID NO:6 (OATP-RP3), SEQ ID NO:8 (OATP-RP4), SEQ ID NO:10 (OATP-RP5), and SEQ ID NO:12 (OATP-RP1).

6. A modified OATP protein comprising an OATP of claim 5 that maintains an activity of said OATP protein of claim 5, wherein said modified OATP protein comprises at least one amino acid substitution or deletion.

7. A method of producing OATP, said method comprising the steps of:

a) inserting a nucleic acid sequence according to claim 1 or 2 encoding said OATP protein, or a homologue thereof, into an appropriate expression vector,

b) transfecting said expression vector into an appropriate transfection host cell,

c) growing said transfected host cells in an appropriate culture media, and

d) purifying the OATP protein, or a homologue thereof, from said culture media.

8. An isolated nucleic acid sequence which hybridizes under stringent conditions to the nucleic acid sequence of claim 1 or 2, wherein said nucleic acid sequence contains at least 18 contiguous nucleotides from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9 or SEQ ID NO:11.

9. An antibody specific for the OATP as claimed in claim 5.

10. The antibody of claim 9 wherein said antibody is a monoclonal antibody.

11. The OATP of claim 5, produced by:

a) inserting a nucleic acid sequence encoding said OATP into an appropriate expression vector,

b) transfecting said expression vector into an appropriate transfection host cell,

c) growing said transfected host cells in an appropriate culture media, and

d) purifying the OATP from said culture media.

12. A method for identifying a ligand which is capable of binding to the OATP of claim 5, or to a part of said OATP, said method comprising the steps of:

(a) reacting said OATP. or part of said OATP, with said ligand which potentially is capable of binding to the OATP or part of said OATP. under conditions which permit the formation of ligand-OATP complexes; and

(b) assaying for ligand-OATP complexes. for free ligand, or for non-complexed OATP.

13. A method for identifying a substrate which is capable of being transported by the OATP of claim 5, or a part of said OATP, said method comprising the steps of:

(a) reacting said OATP, or part of said OATP, with said substrate which is potentially capable of being transported by the said OATP or part of said OATP, under conditions which permit the movement of said substrate across a membrane; and

(b) assaying for the movement of said substrate across the membrane.

14. A method of delivering a molecule to a an organ that expresses an OATP protein of claim 5, said method comprising:

(a) identifying a substrate that is transported by said OATP;

(b) joining said substrate to said molecule to be delivered to form a substrate-molecule fusion compound; and

(c) providing said substrate-molecule fusion compound to said organ.

15. A fusion protein comprising all or a portion of the OATP of claim 5, attached to a second polypeptide.

16. A method for identifying a modulator which is capable of augmenting or inhibiting the transport of a substrate by the OATP of claim 5, or a part of said OATP, said method comprising:

a) reacting said OATP, or part of said OATP, with said substrate and said modulator which potentially is capable of augmenting or inhibiting the transport of a substrate under conditions which permit the movement of said substrate across a membrane;

b) measuring the augmentation or inhibition of transport of said compound by said modulator.

17. A nucleic acid molecule of claim 2, wherein said nucleic acid molecule comprises the OATP gene, or a complement of the OATP gene, contained in ATCC Accession Number 207209.

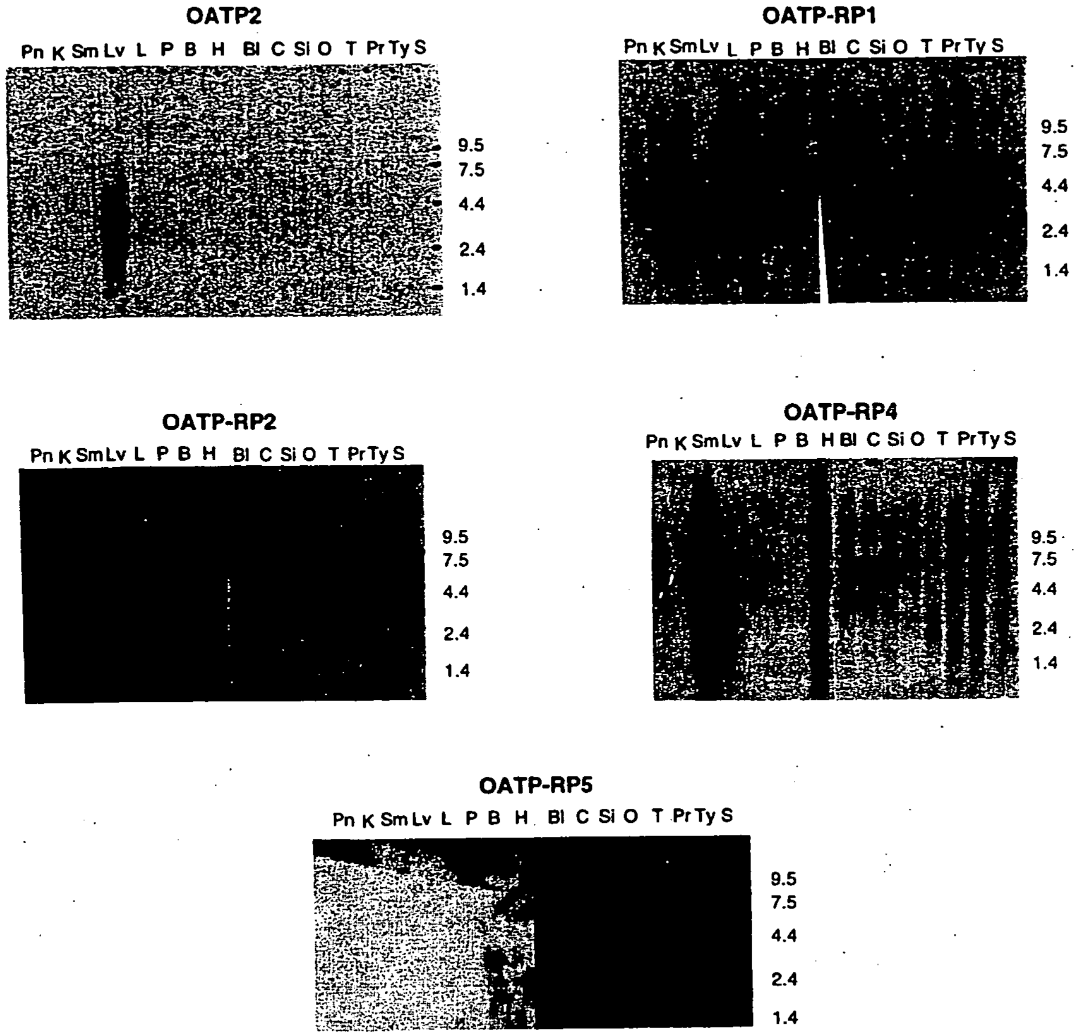
18. A nucleic acid molecule of claim 2, wherein said nucleic acid molecule comprises the OATP gene, or a complement of the OATP gene, contained in ATCC Accession Number 207210.

19. A nucleic acid molecule of claim 2, wherein said nucleic acid molecule comprises the OATP gene, or a complement of the OATP gene, contained in ATCC Accession Number 207211.

20. A nucleic acid molecule of claim 2, wherein said nucleic acid molecule comprises the OATP gene, or a complement of the OATP gene, contained in ATCC Accession Number 207212.

21. A nucleic acid molecule of claim 2, wherein said nucleic acid molecule comprises the OATP gene, or a complement of the OATP gene, contained in ATCC Accession Number 207213.

22. A nucleic acid molecule of claim 2, wherein said nucleic acid molecule comprises the OATP gene, or a complement of the OATP gene, contained in ATCC Accession Number 207214.



Tissue Key

H: heart
B: brain
P: placenta
L: lung
Lv: liver
Sm: skeletal muscle
K: kidney
Pn: pancreas
S: spleen
Ty: thymus
Pr: prostate
T: testis
O: ovary
Si: small intestine
C: colon
Bl: peripheral blood leukocytes

FIG. 1

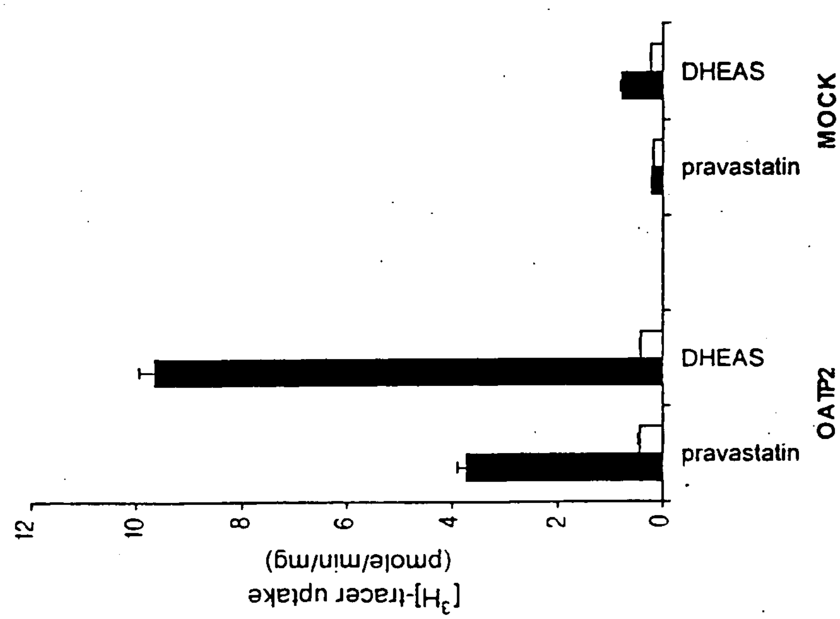


FIG. 2A

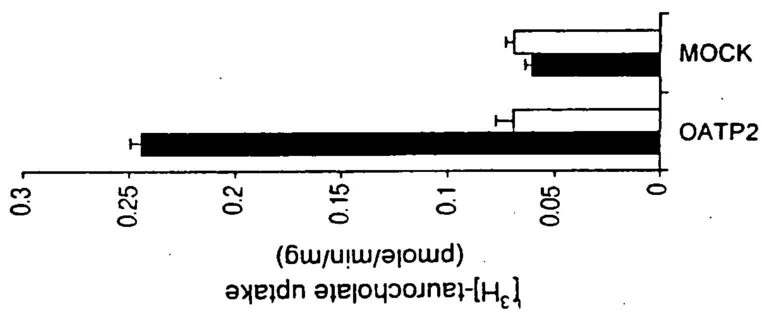


FIG. 2B

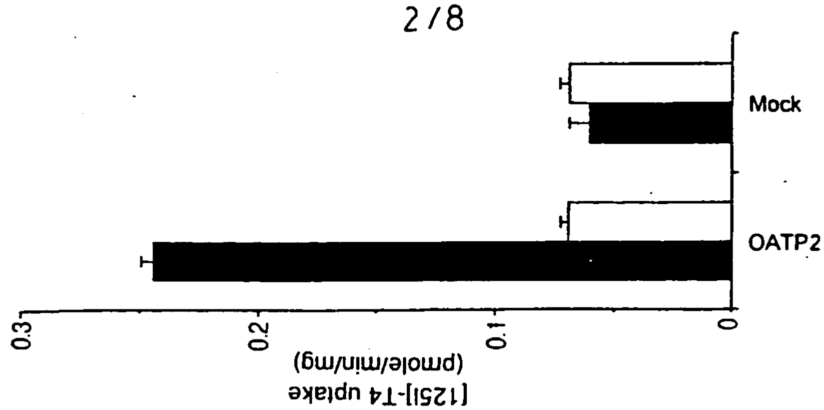


FIG. 2C

```
             1                                                                                     80
roatp2       .......... .......... .......... .......... .......... .......... .......... ..........
roatp3       .......... .......... .......... .......... .......... .......... .......... ..........
rOAT-K1      .......... .......... .......... .......... .......... .......... .......... ..........
roatp1       .......... .......... .......... .......... .......... .......... .......... ..........
hOATP        .......... .......... .......... .......... .......... .......... .......... ..........
hOATP-RP5    .......... .......... .......... .......... .......... .......... .......... .........m
hOATP2       .......... .......... .......... .......... .......... .......... .......... ..........
hOATP-RP3    .......... .......... .......... .......... .......... .......... .......... ..........
hPGT         .......... .......... .......... .......... .......... .......... .......... ..........
hOATP-RP2    .......... .......... .......... .......... .......... .......... .......... ..........
hOATP-RP4    mdegtglqpg ageqleapat aeavqercep etlrskslpv lssascrpsl sptsgdanpa fgcvdssghq elkqgpnpla
hOATP-RP1    .......... .......... mplhqlgdkp ltfpspnsam engldhtpps rraspgtpls pgslrsaahs pldtskqplc
Consensus    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

             81                                                                                   160
roatp2       .......... .........m G........k sEkrvAthg. .vrCFakIKm FLLALtcAyv sksLs.GtYM nSmLTQIERq
roatp3       .......... .........m G........e tEkrvAthe. .vrCFskIKm FLLALtwAyv sqsLs.GiYM ntmLTQIERq
rOAT-K1      .......... .........m G........d lEkgaAthg. .agCFakIKv FLmALtcAyv sksLs.GtfM sSmLTQIERq
roatp1       .......... .........m e........e tEkkiAtqe. .grlFskmKv FLLsLtcAcl tksLs.GvYM nSmLTQIERq
hOATP        .......... .........m G........e tEkriethr. .irClsklKm FLLAitcAfv sktLs.GsYM nSmLTQIERq
hOATP-RP5    dtsskeniql fcktsvq.pv Gr....psfk tEypsseek. .qpCcgelKv FLcALsfvyf akaLa.egYl kStiTQIERR
hOATP2       .......... ..mdqnq.hl n.......kt aEaqpsenkk .trycnglKm FLaALslsfi aktLg.aiiM kSsiihIERR
hOATP-RP3    mqgkkpggss gggrsge.lq G.......de aqrnkkkkkk .vsCFsnIKi FLvsecalml aqgtv.GaYl vSvLTtlERR
hPGT         ........mg llpklgv.sq G.......sd tstsrAgrca .rsvFgnIKv FvLcqgllql cqlLy.saYf kSsLTtIEkR
hOATP-RP2    .......... .mgtent.pg G........k aspdpqdvr. .psvFhnIKl FvLchsllql aqlmi.sgYl kSsistvEkR
hOATP-RP4    pspsapstsa glgdcnh.rv dlsktfsvss alamlqerrc lyvvltdsrc FLvcmcfltf iqaLmvsgYl sSviTtIERR
hOATP-RP1    qlwaekhgar gthevryxsa G......qsv acgwwAfapp clqvlntpKg iLffLcaAaf lqgmtvngfi ntviTslERR
Consensus    ---------- ---------- G--------- -E---A---- ---CF--IK- FLLAL--A-- ---L--G-YM -S-LTQIERR
```

FIG. 3A

```
             1                                                                                80
   roatp2    .......... .......... .......... .......... .......... .......... .......... ..........
   roatp3    .......... .......... .......... .......... .......... .......... .......... ..........
  rOAT-K1    .......... .......... .......... .......... .......... .......... .......... ..........
   roatp1    .......... .......... .......... .......... .......... .......... .......... ..........
    hOATP    .......... .......... .......... .......... .......... .......... .......... ..........
hOATP-RP5    .......... .......... .......... .......... .......... .......... .......... .........m
   hOATP2    .......... .......... .......... .......... .......... .......... .......... ..........
hOATP-RP3    .......... .......... .......... .......... .......... .......... .......... ..........
     hPGT    .......... .......... .......... .......... .......... .......... .......... ..........
hOATP-RP2    .......... .......... .......... .......... .......... .......... .......... ..........
hOATP-RP4    mdegtglqpg ageqleapat aeavqercep etlrskslpv lssascrpsl sptsgdanpa fgcvdssghq elkqgpnpla
hOATP-RP1    .......... .......... mplhqlgdkp ltfpspnsam engldhtpps rraspgtpls pgslrsaahs pldtskqplc
Consensus    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

             81                                                                              160
   roatp2    .......... .........m G........k sEkrvAthg. .vrCFakIKm FLLALtcAyv sksLs.GtYM nSmLTQIERq
   roatp3    .......... .........m G........e tEkrvAthe. .vrCFskIKm FLLALtwAyv sqsLs.GiYM ntmLTQIERq
  rOAT-K1    .......... .........m G........d lEkgaAthg. .agCFakIKv FLmALtcAyv sksLs.GtfM sSmLTQIERq
   roatp1    .......... .........m e........e tEkkiAtqe. .grlFskmKv FLLsLtcAcl tksLs.GvYM nSmLTQIERq
    hOATP    .......... .........m G........e tEkriethr. .irClsklKm FLLAitcAfv sktLs.GsYM nSmLTQIERq
hOATP-RP5    dtsskeniql fcktsvq.pv Gr....psfk tEypsseek. .qpCcgelKv FLcALsfvyf akaLa.egYl kStiTQIERR
   hOATP2    .......... ..mdqnq.hl n.......kt aEaqpsenkk .trycnglKm FLaALslsfi aktLg.aiiM kSsiihIERR
hOATP-RP3    mqgkkpggss gggrsge.lq G.......de aqrnkkkkkk .vsCFsnIKi FLvsecalml aqgtv.GaYl vSvLTtlERR
     hPGT    ........mg llpklgv.sq G.......sd tstsrAgrca .rsvFgnIKv FvLcqgllql cqlLy.saYf kSsLTtIEkR
hOATP-RP2    .......... .mgtent.pg G........k aspdpqdvr. .psvFhnIKl FvLchsllql aqlmi.sgYl kSsistvEkR
hOATP-RP4    pspsapstsa glgdcnh.rv dlsktfsvss alamlqerrc lyvvltdsrc FLvcmcfltf iqaLmvsgYl sSviTtIERR
hOATP-RP1    qlwaekhgar gthevryxsa G......qsv acgwwAfapp clqvlntpKg iLffLcaAaf lqgmtvngfi ntviTslERR
Consensus    ---------- ---------- G--------- -E---A---- ---CF--IK- FLLAL--A-- ---L--G-YM -S-LTQIERR
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FIG. 3A

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             161                                                                                                240
roatp2       FgIptSiVGL InGSFEIGNL LLIiFVSYFG tKLHRPimIG vGCavMGLGc fLisLPHFLM GqYEYEt... ilptsNvsSn
roatp3       FdIpiSiVGf InGSFEIGNf LLIiFVSYFG tKLHRPimIG vGCviMGLGc fLmsLPHFLM GrYEYEtt.. isptsNlsSn
rOAT-K1      FgIptaiVGf InGSFEIGNL LLIiFVSYFG mKLHRPivIG vGCavMGLGc fiisLPHFLM GrYEYEtt.. ilptsNlsSn
roatp1       FdIstSvaGL InGSFEIGNL ffIvFVSYFG tKLHRPvvIG iGCviMGLGc lLmsLPHFfM GrYEYEtt.. isptgNlsSn
hOATP        FnIptSlVGf InGSFEIGNL LLIiFVSYFG tKLHRPimIG iGCvvMGLGc fLksLPHFLM nqYEYEst.. vsvsgNlsSn
hOATP-RP5    FdIpSSlVGv IdGSFEIGNL LvItFVSYFG aKLHRPkiIG aGCviMGvGt lLiamPqFfM eqYkYEr... yspssNstls
hOATP2       FeIsSSlVGf IdGSFEIGNL LvIvFVSYFG sKLHRPklIG iGCfiMGiGg vLtaLPHFfM GyYrYsketn idsseNstSt
hOATP-RP3    FnlqSadVGv IasSFEIGNL aLIlFVSYFG argHRPrlIG cGgivMaLGa lLsaLPeFLt hqYkYEag.. .eirwgaegr
hPGT         FglsSSssGL IsslnEIsNa iLIiFVSYFG srvHRPrlIG iGglflaaGa filtLPHFLs epYqYtla.. .stgnNsrlq
hOATP-RP2    FglsSqtsGL lasfnEvGNt aLIvFVSYFG srvHRPrmIG yGailvaLag lLmtLPHFis epYrYdnts. .pedmpqdfk
hOATP-RP4    yslkSSesGL lvscFdIGNL vvvvFVSYFG grgrRPlwla vGglliafGa aLfaLPHFis ppYqiqe... ..lnasapnd
hOATP-RP1    ydlhSyqsGL IasSydIaac LcltFVSYFG gsgHkPrwlG wGvllMGtGs lvfaLPHFta GrYEvEl... .....dagvr
Consensus    F-I-SS-VGL I-GSFEIGNL LLI-FVSYFG -KLHRP--IG -GC--MGLG- -L--LPHFLM G-YEYE---- -----N--S-

             241                                                                                                320
roatp2       sffCveNrSq .....tLnPt qD..psECvK Emk.SLMWIY VlVG...NIi RGIGETPImP LGISYIeDFA KsENSPLYIG
roatp3       sfLCmeNrSq .....tLkPt qD..paECiK Emk.SLMWIY VlVG...NIi RGIGETPImP LGISYIeDFA KsENSPLYIG
rOAT-K1      sfLCmeNqtq .....tLnPa qD..paECvK Evk.SLMWIY VlVG...NIi RGIGETPImP LGvSYIenFA KsENSPLYIG
roatp1       sfLCmeNrtq .....tLkPt qD..paECvK Emk.SLMWIc VmVG...NIi RGIGETPIvP LGISYIeDFA KsENSPLYIG
hOATP        sfLCmeNgtq .....iLrPt qD..psECtK Evk.SLMWvY VlVG...NIv RGmGETPIlP LGISYIeDFA KfENSPLYIG
hOATP-RP5    ispCllesSs qlpvsvmeks kskisnECev dts.SsMWIY VflG...NlL RGIGETPIqP LGIaYlDDFA sedNaafYIG
hOATP2       lstCliNqil s....lnras peivgkgClK Esg.SyMWIY VfmG...NmL RGIGETPIvP LGlSYIDDFA KeghSsLYlG
hOATP-RP3    .dvCaaNgSg g....degPd pD...liCrn rta.tnMmyl lliGa..qvL lGIGaTPvqP LGvSYIDDhv rrkdSsLYIG
hPGT         aeLCqkhwqd ......LpPs kchsttqnpq ket.SsMWgl mvVa...qlL aGIGtvPIqP fGISYvDDFs epsNSPLYIs
hOATP-RP2    asLClpttSa p....asaPs ng..ncssyt Etq.hLsvvg imfva..qtL lGvGgvPIqP fGISYIvDFA hnsNSPLYlG
hOATP-RP4    .gLCqggnSt a....tLeP. p.....aCpK dsggnnhWvY lalficaqIL iGmGsTPIyt LGptYlDDnv KkENSsLYla
hOATP-RP1    ..tCpaN... ........P. ....gavCad stsglsryql Vfmlg..qfL hGvGaTPlyt LGvtYlDenv KsscSPvYIa
Consensus    --LC--N-S- ------L-P- -D----EC-K E---SLMWIY V-VG---NIL RGIGETPI-P LGISYIDDFA K-ENSPLYIG
```

FIG. 3B

```
            321                                                                                        400
roatp2      ILeTgmtiGP liGlLLaSsC AnIYVDiesV NTDdLtITPt DtRWVGAWWi GFLvCAGvni LtSfPFFFFP KtLP...KeG
roatp3      ILeTgkvfGP ivGlLLGSfC AsIYVDtGsV NTDdLtITPt DtRWVGAWWi GFLiCAGvni LsSIPFFFFP KtLP...KeG
rOAT-K1     ILeTgkmiGP ifGlLLGSfC AsIYVDtGsV NTDdLtITPt DiRWVGAWWi GFLvCAGvni LiSIPFFFFP KtLP...KeG
roatp1      ILemgkvaGP ifGlLLGSyC AqIYVDiGsV NTDdLtITPs DtRWVGAWWi GFLvCAGvni LtSIPFFFlP KaLP...KkG
hOATP       lveTgaiiGP liGlLLaSfC AnvYVDtGfV NTDdLiITPt DtRWVGAWWf GFLiCAGvnv LtaIPFFFlP ntLP...KeG
hOATP-RP5   cvqTvaiiGP ifGfLLGSlC AklYVDiGfV NlDhitITPk DPqWVGAWWl GyLiagiisl LaavPFwylP KsLP...rsq
hOATP2      ILnaiamiGP iiGftLGSlf skmYVDiGyV dlstirITPt DsRWVGAWWl nFLvsglfsi isSIPFFFlP qtpn...Kpq
hOATP-RP3   ILfTmlvfGP acGfiLGSfC tkIYVDavfi dTsnLdITPd DPRWiGAWWg GFLlCgallf fsSllmFgFP qsLPphsdpa
hPGT        ILfaisvfGP afGyLLGSim lqIfVDyGrV NTaavnlvPg DPRWiGAWWl GlLissallv LtSfPFFFFP ramP....iG
hOATP-RP2   ILfavtmmGP glafgLGSlm lrlYVDinqm peggislTik DPRWVGAWWl GFLiaAGava LaaIPyFFFP KemPkekrel
hOATP-RP4   ImyvmgalGP avGyLLGgll igfYVDp... .rnpvhldqn DPRfiGnWWs GFLlCAiamf LvifPmFtFP KkLPprhKkk
hOATP-RP1   IfyTaailGP aaGyLiGgal lnIYtemG.. ..rrtelTte sPlWVGAWWv GFLgsgaaaf ftavPilgyP rqLP....gs
Consensus   IL-T----GP --G-LLGS-C A-IYVD-G-V NTD-L-ITP- DPRWVGAWW- GFL-CAG--- L-SIPFFFFP K-LP---K-G

            401                                                                                        480
roatp2      lq..enVdgt e........n akekkhrkka k......... ...eekrgit KDFfvfmKsL scNPiYmLfi LisVlQfNaf
roatp3      lq..ddVdgt n........n dkeekhreka k......... ...eenrgit KDFlpfmKsL scNPiYmLli LtsVlQiNaf
rOAT-K1     lq..enVdgt e........n akeestekrp r......... ...kknrgit KDFfpflKsp vlqPdlhavh pykVlQvNaf
roatp1      qq..enVavt k........d gkvekyggqa r......... ...eenlgit KDFltfmKrL fcNPiYmLfi LtsVlQvNgf
hOATP       le..tnadii k........n enedkqkeev k......... ...kekygit KDFlpfmKsL scNPiYmLfi LvsViQfNaf
hOATP-RP5   sr..edsnss sekskfii.d dhtdyqtpqg en........ ...akimema rDFlpslKnL fgNPvYfLyl ctstvQfNsl
hOATP2      ke..rkasls lhvletn..d ekdqtanltn qg........ ...knitknv tgFfqsfKsi LtNPlYvmfv LltllQvssy
hOATP-RP3   mes.eqamls ereyerpkps ngvlrhplep dss....... ...ascfqql rvipkvtKhL LsNPvftcii Laacmeiavv
hPGT        ak...rapat a........d earkleeaks r......... ...gslvdfi KrFpciflrL LmNslfvLvv Laqctfssvi
hOATP-RP2   qfr.rkVlav tdsparkgkd spskqspges tkkqdglvqi apnltviqfi KvFprvllqt LrhPiflLvv LsqVclssma
hOATP-RP4   kkkkfsVdav sdddvlkeks nnseqadkkv ss........ ...mgfgkdv rDlpraavri LsNmtflfvs Lsytaesaiv
hOATP-RP1   qr..yaVmra a..em....h qlkdssrgea sn........ ...pdfgkti rDlplsiwlL LkNPtfiLlc Lagateatli
Consensus   ------V--- ---------- ---------- ---------- ---------- KDF----K-L L-NP-Y-L-- L--V-Q-N--
```

FIG. 3C

```
            481                                                                                                  560
roatp2      insfTFmPKY LEQQYGkSta evvFLmGlym LPpiClGyli GGlIMKKFKv tVkkAAhLAf wlcLseYLLs flsyvmtCdN
roatp3      inmfTFLPKY LEQQYGkSta evvlLiGvyn LPpiCiGyll iGfIMKKFKi tVkkAAymAf clSLfeYLLy flhFmitCdN
rOAT-K1     niyfsFLPKY LEnQYGkSta eviFLmGvyn LPaiCiGyli aGfmMKKFKi tVktAAfLrf clSLseYsfg fcnFlitCdN
roatp1      inkfTFLPKY LEQQYGkSta eAiFLiGvys LPpiClGyli GGfIMKKFKi tVkkAAyLAf clSvfeYLLf lchFmltCdN
hOATP       vnmisFmPKY LEQQYGiSsS dAiFLmGiyn LPpiCiGyii GGlIMKKFKi tVkqAAhigc wlSLleYLLy flsFlmtCeN
hOATP-RP5   fGmvTykPKY iEQQYGqSsS rAnFviGlin iPavalGifs GGivMKKFri sVcgAAkLyl gsSvfgYLLf lslFalgCeN
hOATP2      iGafTyvfKY vEQQYGqpsS kAniLlGvit iPifasGmfl GGyIiKKFKl ntvgiAkfsc ftavmslsfy llyFfilCeN
hOATP-RP3   aGfaaFLgKY LEQQfnlttS sAnqLlGmta iPcaClGifl GGllvKKlsl salgAirmAm lvnLvstacy vsflflgCdt
hPGT        aGlsTFLnKf LEkQYGtSaa yAnFLiGavn LPaaalGmlf GGilMKrFvf slqtipriAt tiitismiLc vplFfmgCst
hOATP-RP2   aGmaTFLPKf LErQfsitaS yAnlLiGcls fPsvivGivv GGvlvKrlhl gpvgcgaLcl lgmLlclffs lplFfigCss
hOATP-RP4   tafiTFiPKf iEsQfGipaS nAsiytGvii vPsagvGivl GGyIiKKlKl garesAkLAm icSgvslLcf stlFivgCes
hOATP-RP1   tGmsTFsPKf LEsQfslSaS eAatLfGylv vPagggGtfl GGffvnKlrl rgsavikfcl fctvvslLg. ilvFslhCps
Consensus   -G--TFLPKY LEQQYG-S-S -A-FL-G--- LP--C-G--- GG-IMKKFK- -V--AA-LA- --SL--YLL- ---F---C-N

            561                                                                                                  640
roatp2      fpVAGLTtSY eGvqhqlyvE nkvlADCNtr CnCstntWdP VCG.dNGlaY mSACLAGCe. ..kSvGTGtN mVFq.NCSCI
roatp3      fpVAGLTalY eGvhhplyvE nkvlADCNrg CSCstnsWdP VCG.dNGlaY mSACLAGCk. ..kSvGTGtN mVFq.NCSCI
rOAT-K1     vpVAGLTnSY erdqkplylE nnvlADCNtr CSCltktWdP VCG.dNGlaY mSACLAGCe. ..kSvGTGtN mVFh.NCSCI
roatp1      aaVAGLTtSY kGvqhqlhvE skvlADCNtr CSCstntWdP VCG.dNGvaY mSACLAGCk. ..kfvGTGtN mVFq.dCSCI
hOATP       ssVvGintSY eGipqdlyvE ndifADCNvd CnCpskiWdP VCG.nNGlsY lSACLAGCe. ..tSiGTGiN mVFq.NCSCI
hOATP-RP5   sdVAGLTvSY qGtkpvsyhE ralfsDCNsr CkCsetkWeP mCG.eNGitY vSACLAGCq. ..tSnrsGxN iiFy.NCtCv
hOATP2      ksVAGLTmtY dGnnpvtshr dvplsyCNsd CnCdesqWeP VCG.nNGitY iSpCLAGCk. ..sSsGnkkp iVFy.NCSCl
hOATP-RP3   gpVAGvTvpY .Gnstapgsa ldpyspCNnn CeCqtdsftP VCG.adGitY lSACfAGCn. ..stnlTG.. ......CaCl
hPGT        ptVAevypps ..tss.sihp qs..paCrrd CSCpdsifhP VCG.dNGieY lSpChAGCsn inmSsaTskq liyl.NCSCv
hOATP-RP2   hqiAGiThqt ...sahpglE ls..psCmea CSCpldgfnP VCdpstrveY itpChAGCss wvvqdaldns qVFytNCSCv
hOATP-RP4   inlgGinipY ttgpsltmph rnltgsCNvn CgCkiheyeP VCG.sdGitY fnpCLAGCv. ..nSgnlstg irnyteCtCv
hOATP-RP1   vpmAGvTaSY .Ggsllpegh lnltApCNaa CSCqpehysP VCG.sdGlmY fSlChAGCpa atetnvdGqk .Vyr.dCSCI
Consensus   --VAGLT-SY -G-------E ----ADCN-- CSC----W-P VCG--NG--Y -SACLAGC-- ---S-GTG-N -VF--NCSCI
```

FIG. 3D

```
             641                                                                                      720
roatp2       qs........ sGNSS..... .......... .AVLGlCnKg PdCankLqYF LiiaifgcFI ySLagIPGYM VlLRCiKsEE
roatp3       rs........ sGNSS..... .......... .AVLGlCkKg PeCankLqYF LimsvigSFI ySitaIPGYM VlLRCiKpEk
rOAT-K1      qs........ pGNSS..... .......... .AVLGlCnKg PeCtnkLqYl LilsgflSil ySfaaIPGYM VfLRCiKsEE
roatp1       qs........ lGNSS..... .......... .AVLGlCkKg PeCanrLqYF LiltiiiSFI ySLtaIPGYM VfLRCVKsEE
hOATP        qt........ sGNSS..... .......... .AVLGlCdKg PdCslmLqYF LilsamsSFI ySLaaIPGYM VlLRCmKsEE
hOATP-RP5    gia....ask sGNSS..... .......... .givGrCqKd ngCpqmflYF LvisvitSyt lSLggIPGYi llLRCiKpql
hOATP2       evt....glq nrNyS..... .......... .AhLGeCprd daCtrkfyfF vaiqvlnlFf saLggtshvM livkiVqpEl
hOATP-RP3    ttv....p.. aeNat..... .......... .vVpGkCp.s PgCqeafltF LcvmcicSlI gamaqtPsvi iliRtVspEl
hPGT         tg........ .GsaS..... .......... .AktGsCp.. vpCahfLlpa iflisfvSlI acishnPlYM mvLRvVnqEE
hOATP-RP2    ve........ .GNp...... .......... .vlaGsCd.. stCshlvvpF LllvslgSal acLthtPsfM liLRgVKkEd
hOATP-RP4    qsrqvitppt vGqrSqlrvv ivktylneng yAVsGkCkr. .tCntlip.F LvflfivtFI tacaqpsaii VtLRsVedEE
hOATP-RP1    pqn.....ls sGfgh..... .......... .AtaGkCt.. stCqrkpl.l LvfifvviFf tfLssIPalt atLRCVrdpq
Consensus    ---------- -GNSS----- ---------- -AVLG-C-K- P-C---L-YF L------SFI -SL--IPGYM V-LRCVK-EE

             721                                                                                      800
roatp2       KSLgvGlHaf ciRiLAGIPA PIYFGALIDr TCLHWGTlkC GepGACRmYD insFRrlYLG LpaALRgaSf vpaffILrLt
roatp3       KSLgiGlHaf ctRvfAGIPA PIYFGALIDr TCLHWGTlkC GepGACRmYn innFRriYLv LpaALRgsSy LpalfILiLm
rOAT-K1      KSLgiGiHaf ciRvfAGIPA PIYFGALIDr TCLHWGTqkC GapGr.RmYD insFRriYLG msaALRgsSy LpafvIviLt
roatp1       KSLgvGlHtf ciRvfAGIPA PvYFGALIDr TCLHWGTlkC GqrGACRmYD insFRhiYLG LpiALRgsSy LpaffILiLm
hOATP        KSLgvGlHtf ctRvfAGIPA PIYFGALmDs TCLHWGTlkC GesGACRiYD sttFRyiYLG LpaALRgsSf vpaliILiLl
hOATP-RP5    KSfAlGiytl aiRvLAGIPA PvYFGvLIDt sCLkWGfkrC GsrGsCRlYD snvFRhiYLG LtviLgtvSi LlsiavLfil
hOATP2       KSLAlGfHsm viRaLgGIlA PIYFGALIDt TCikWsTnnC GtrGsCRtYn stsFsrvYLG LssmLRvsSl vlyiiliyam
hOATP-RP3    KSyAlGvlfl llRlLgfIPp PliFGAgIDs TCLfWsT.fC GeqGACvlYD nvvyRylYvs iaiALksfaf ilytttwqcl
hPGT         KSfAiGvqfl lmRlLAwlPs PalyGltIDh sCirWnslcl GrrGACayYD ndalRdrYLG Lqmgykalgm LllcfIswrv
hOATP-RP2    KtLAvGiqfm flRiLAwmPs PvihGsaIDt TCvHWal.sC GrravCRyYn ndllRnrfiG Lqfffktgsv icfalvLavl
hOATP-RP4    rpfAlGmqfv llRtLAyIPt PIYFGAvIDt TCmlWqq.eC GvqGsCweYn vtsFRfvYfG LaagLkfvgf ififlawysi
hOATP-RP1    rSfAlGiqwi vvRiLgGIPg PIaFGwvIDk aCLlWqd.qC GqqGsClvYq nsamsryiLi mgllykvlgv LffaiacfLy
Consensus    KSLA-G-H-- --R-LAGIPA PIYFGALID- TCLHWGT--C G--GACR-YD ---FR--YLG L--ALR--S- L----IL-L-
```

FIG. 3E

```
             801                                                                                        880
    roatp2   R......tfq fPgd...IeS Sk....tdha emkltlKESe cTevlrs.kv t..eD..... .......... ..........
    roatp3   R......Kfq fPge...IdS SE....tela emkitvKkSe cTdvHgspqv e..nDgElkT rl........ ..........
   rOAT-K1   R......Kfs lPgk...InS SE....meia emklteKESq cTdvHrnpkf k..nDgElkT kl........ ..........
    roatp1   R......Kfq fPgd...IdS Sa....tdht emmlgeKESe hTdvHgspqv e..nDgElkT kl........ ..........
     hOATP   R......Kch lPge...naS Sg....teli etkvkgKEne ckdiyqkstv l..kDdElkT kl........ ..........
 hOATP-RP5   k......Kny vskhrsfItk rE....rtmv strfq.KEny tTsdHllqpn y..wpgke.T ql........ ..........
    hOATP2   k......Kky qekd...Ina SE....ngsv mdean.lESl nknkHfvpsa g..aDsEthc .......... ..........
 hOATP-RP3   Rkny...Kry iknhegglst SEff.astlt ldnlgrdpvp anqtHrtkfi ynleDhEwce nmesvl.... ..........
      hPGT   k......Knk .eyn...vqk aa.....gli .......... .......... .......... .......... ..........
 hOATP-RP2   Rqqd...Kea rtke...srS Sp.....ave qqllvsgpgk kpedsrv... .......... .......... ..........
 hOATP-RP4   kykedglqrr rqrefplstv SErvghpdna rtrscpafSt qgefHeetgl qkgiqcaaqT ypgpfpeais ssadpglees
 hOATP-RP1   k......... .Pls....eS Sd...gletc lpsqssapds aTdsqlqssv .......... .......... ..........
 Consensus   R------K-- -P-----I-S SE-------- ------KES- -T--H----- ----D-E--T ---------- ----------

             881
    roatp2   ........
    roatp3   ........
   rOAT-K1   ........
    roatp1   ........
     hOATP   ........
 hOATP-RP5   ........
    hOATP2   ........
 hOATP-RP3   ........
      hPGT   ........
 hOATP-RP2   ........
 hOATP-RP4   paalepxs
 hOATP-RP1   ........
 Consensus   --------
```

FIG. 3F

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(72) Inventors: KIRCHGESSNER, Todd, G.; 2107 Goodwin Lane, North Wales, PA 19454 (US). HSIANG, Bonnie; 76 Stanford Road East, Trenton, NJ 08638 (US). ZHU, Yingjie; 12 Lafata Lane, Killingworth, CT 06419 (US). WU, Yuli; 3 Susanna Way, Newtown, PA 18940 (US). WANG, Zhaoqing; 55 Marvin Lane, Piscataway, NJ 08854 (US). LYNCH, Jean, S.; 26 Welisewitz Road, Ringoes, NJ 08551 (US). HUANG, Xin; 210 Princeton Arms North, Cranbury, NJ 08512 (US). YANG, Wen-Pin; 25 Rutgers Lane, Princeton, NJ 08540 (US).

(74) Agents: KLEIN, Christopher et al.; Bristol-Myers Squibb Company, P.O. Box 4000, Princeton, NJ 08543-4000 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/71566 A3

(54) Title: NOVEL ORGANIC ANION TRANSPORT PROTEINS

(57) Abstract: The current invention discloses nucleic acid and amino acid sequences for novel organic anion transfer proteins ("OATPs"). The invention encompasses the OATPs described herein, together with vectors containing the cDNA sequences, host cells containing the vectors and polypeptides having all or part of an OATP. Also encompasses are uses for OATPs for targeting drugs to specific organs and for modulating the concentration of endogenous substrates.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/13939

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JACQUEMIN et al. Expression cloning of a rat liver Na+-independent organic anion transporter. Proc. Natl. Acad. Sci. USA. January 1994, Vol. 91, pages 133-137.	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 SEPTEMBER 2000	14 NOV 2000

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Dorthea Lawrence for PREMA MERTZ
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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International application No.
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A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07K 14/435, 16/18; A61K 38/16,38/17; G01N 33/53, 33/567; C12N 5/10, 15/12, 15/63, 15/64

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/350, 387.1, 387.9, 388.1; 536/23.1, 23.5, 24.3, 24.31; 435/69.1, 471, 71.1, 71.2, 320.1, 325, 252.3, 254.11 , 7.1, 7.2; 514/2, 8, 12

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

530/350, 387.1, 387.9, 388.1; 536/23.1, 23.5, 24.3, 24.31; 435/69.1, 471, 71.1, 71.2, 320.1, 325, 252.3, 254.11 , 7.1, 7.2; 514/2, 8, 12

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, CAS ONLINE, MEDLINE, CAPLUS
search terms: organic anion transport protein, human OATP, nucleic acid, recombinant protein, production, antibodies, fusion proteins, ligands, modulators, agonists, antagonists, method, assay, treatment, therapy, administer.